BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

REMARKS/ARGUMENTS

After entry of this amendment, claims 11, 14, 16, 18, 19, 21-25, 58-71 are pending. Claims 11, 14, 16, 18, 19, 21-25, and 59-71 are under consideration, claims 1-10, 12, 13, 15, 17, 20, and 26-57 having been canceled, and new claims 59-71 having been added.

The term "preventing" has been replaced with "prophylaxis" in the claims. Previous claim 11 referred to "treating" and "preventing" in the same claim. Because "prophylaxis" is a noun and 'treating" is a verb, it is grammatically awkward to refer to both in the same claim. Accordingly, treating and prophylaxis are now claimed in two independent claims 11 and 59, respectively. New independent claim 59 corresponds to claim 11. Claims 60-69, which depend from claim 59, correspond to claims 11, 14, 16, 18, 19, and 21-25, respectively. Support for new claims 70 and 71 is provided at *e.g.*, p. 50, line 11. Support for prophylaxis is provided at *e.g.*, p. 51, lines 17-19. Applicant addresses the Examiner's comments using the paragraph numbering of the office action.

¶2. As noted in the previous response, the restriction requirement is moot in view of amendments to the claims.

¶¶13-27. The Examiner maintains the rejection of claims 11, 14, 15, 18, 19, 21-25 under 35 USC 112, first paragraph. The Examiner alleges the claims are enabled only for treatment of Alzheimer's disease comprising administering AB42. Although many of the previous bases for rejection have been withdrawn, it appears that three principal issues remain, which will be addressed in turn.

First, the Examiner alleges that "prevention" means an "all or nothing" effect rather than a lowered incidence of disease or alleviation of symptoms (¶¶15-18). In the context of preventive medicine, it is submitted that a regime does not have to achieve complete and total stoppage of disease in every patient to be considered a preventive treatment. Nevertheless, to speed prosecution, applicants have replaced reference to prevention in the claims with "prophylaxis."

Second, the Examiner alleges that the evidence provided in the last response (*i.e.*, the declaration of Martin Koller M.D., M.P.H.) does not show that the claimed method would have any effect on Down's syndrome, hereditary cerebral hemorrhage, sporadic cerebral amyloid angiopathy or inclusion body myositis or Down's syndrome.

Insofar as the Examiner is alleging the human clinical testing is required for indications other than Alzheimer's disease analogous to the clinical trials described for Alzheimer's disease in the Koller declaration, it is submitted that too high a standard of enablement is being imposed. "Title 35 does not demand that such human testing occur within the confines of Patent and Trademark Office (PTO) proceedings." *In re Brana*, 34 USPQ2d 1436, 1442 (Fed. Cir. 1995). The burden is on the PTO to show lack of enablement, not for the applicant to prove enablement. *In re Marzocci*, 169 USPQ 367, 370 (CCPA 1971). If the evidence is in "equipoise," an inventor is "entitled to a patent." *In re Oetiker,* 24 USPQ2d 1443, 1447 (Fed. Cir. 1992) (Plager, J., concurring).

Here, the Examiner has not addressed the explanation provided in the last response and amplified below why the claimed methods would be expected to have activity against other diseases characterized at least in part by similar pathology to Alzheimer's disease, *i.e.*, amyloid deposits of AB. In each case, one would expect that induced antibodies to Aβ would act to prevent further deposition of Aβ and/or clear existing deposits. In each case, one would expect that inhibiting or clearing the underlying pathology would have a similar effect on clinical symptoms of disease that result from the underlying pathology.

The other diseases mentioned by the Examiner are characterized at least in part by similar pathology to Alzheimer's disease (*see, e.g.*, for Down's syndrome, Hyman, *Prog. Clin. , Biol. Res.,* 379, 123-42 [1992]; and Brugge et al., *Neurology*, 44, 232-8 [1994]; for hereditary cerebral hemorrhage with amyloidosis-Dutch type *see, e.g.*, Rozemuller et al., *Am. J. Pathol.*, 142 [5], 1449-57 [1993]; for inclusion body myositis *see, e.g.*, Askanas et al., *Am. J. Pathol.*, 141[1]:31-36 [1992]; and, for familial cerebral amyloid angiopathy *see, e.g.*, Revesz et al., *Brain Pathol.*, 12, 343-57 (2002) (copies of which are attached). In the case of Down's syndrome, for example, the similarity in pathology with Alzheimer's disease can be explained by the trisomy of chromosome 21 on which the gene encoding amyloid precursor protein resides. Extra

production of this protein due to the extra copy of the gene can lead to extra production of its proteolytic processing product Aβ and hence deposition in to plaques (*see* Hyman). Because Down's syndrome and other cognitive impairment are characterized at least in part by similar pathology (*i.e.*, amyloid deposits of Aβ), the skilled person has reason is reason to expect that a treatment that is effective against Alzheimer's disease is also at least somewhat effective against Down's syndrome. The same expectation applies to other diseases having similar pathology.

It is immaterial that some of the above diseases may have additional pathologies not susceptible to treatment by the claimed methods. For example, in Down's syndrome, trisomy of chromosome 21 may result in additional pathologies besides Aβ deposits. The present methods obviously cannot remove the underlying trisomy. However, there is every reason to expect that amyloid deposits of Aβ in the brain of a patient contribute to symptoms of disease in Down's syndrome as they do in Alzheimer's disease, and that treatment that reduces accumulation or inhibits further accumulation of these deposits in a Down's syndrome patient would be of benefit to the Down's patient as to the Alzheimer's patient. The claims as presently formulated do not require that the treatment completely eradicate or completely prevent the disease. Thus, it is immaterial that there may be some components of Down's syndrome or other disease characterized by amyloid deposits of Aβ may remain after treatment. Rather, it is sufficient that the methods result in some benefit in some of these patients.

Third, the Examiner alleges that the specification does not provide guidance or examples that would enable use of fragments other than Aβ42 (¶24). The Examiner alleges that deletions, insertions or substitutions of amino acids can lead to structural and functional changes in biological activity and immunological properties (citing to Skolnick & Fetrow and Jobling & Holmes). The Examiner alleges that biological function and immunological recognition are unpredictable properties which must be experimentally determined. The Examiner also says that for small peptides, conjugation appears to be required for promoting an effective immune response.

Insofar as the Examiner is alleging the methods are enabled only for Aβ42 because this was the only agent tested in clinical trial described in the Koller declaration, he is again applying too high a standard of enablement. As discussed above, human clinical testing is

not needed for enablement of a therapeutic method, and particularly not a separate human clinical trial for each agent encompassed by the method. The PDAPP mouse model used in the Examples of the specification is an accepted model of Alzheimer's disease as discussed at length in the previous response. The Examiner apparently agrees with this position (office action at ¶19), yet has not addressed the evidence provided by this model regarding selection of appropriate agents (see previous response at pp. 21-22).

Although the Examiner lists the Wands factors relevant to enablement, he appears to give undue emphasis to the alleged unpredictability of mutations on protein structure and function. The effect of mutations may in some instances be unpredictable, but this is not detrimental to enablement if it can be determined which agents have the desired effect by even a considerable amount of experimentation that is routine or based on the guidance of the specification.

> [E]xperimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art...The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.
>
> *In re Wands*, 8 USPQ2d 1400, 858 F.2d 731, 737 (Fed. Cir. 1988).

The issue in *Wands* was whether the specification of the *Wands* patent enabled production of a class of antibodies having IgM isotype and a binding affinity of at least 10^9 M^{-1} using Kohler Milstein technology. As the Examiner is aware, Kohler Milstein technology is a classical technique that involves individualized screening of hybridomas to identify a subset with desired binding characteristics. Until the hybridomas have been screened, it is unpredictable which will have the desired characteristics. Nevertheless, the court found that "practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody" (858 F.2d at 740). The *Wands* patent was held to be enabled.

Here, the specification provides considerable guidance as to which fragments have the desired activity, and how to screen additional agents for the desired activity. First, the specification describes an example (*see* Example IV at p. 75 *et seq.*) in which Aβ1-5 was shown to reduce Aβ deposits in the cortex at statistically significant levels. Second, the specification provides data that three different monoclonal antibodies binding to epitopes 1-5, 3-6 or 3-7 bound to and phagocytosed amyloid deposits (*see* Example XI at p. 95 *et seq.*). One would expect that N-terminal fragments including epitopes 1-5, 3-6 or 3-7 would generate antibodies having similar specificity to the monoclonals found to induce phagocytosis amyloid deposits, and would therefore achieve similar results. Third, a related application published as WO 00/72880[1] provides data mapping the epitope specificity of antibodies induced by immunization with Aβ1-42 (*see* Example XVII at p. 101 *et seq.*). The data indicate the most of the antibodies bind to epitopes within Aβ1-11. Collectively, these experiments show that fragments containing N-terminal epitopes can achieve a clearing response against amyloid deposits in similar fashion to Aβ42.

Further, other fragments could be screened using the same methods and same endpoints. The number of fragments of Aβ is not infinite and because many of the various possible fragments of beta amyloid peptide have overlapping sequences, the key regions of peptide needed for pharmacological activity can be determined by screening only a relatively small proportion of the total number of peptides. For example, if it is found that deletion of 20 amino acids from the C-terminus has no lowering of activity, then one can infer that deletions of fewer amino acids from the C-terminus will also not lower activity. Thus, by testing only a few of the possible fragments of beta amyloid peptide, one can predict whether any other fragment will have pharmacological activity. Other agents could be screened using the same transgenic animal model with an initial preliminary screen to show the antibody can elicit antibodies that bind to Aβ (*see* specification at paragraph bridging pp. 31-32).

With respect to carrier molecules, the specification provides general guidance that carriers are likely to be required for shorter peptides (*see* specification at p. 44. lines 5-7). The specification also provides particular examples in which Aβ42 was found to be effective without a carrier and Aβ1-5 with a carrier (*see* specification at p. 82). With intermediate fragments sizes,

[1] Cited as cite no. 322 in the Supplemental IDS filed on May 27, 2003 for instant application.

it would be a routine matter to determine whether a carrier is required. For example, one could perform an initial screen with a carrier. If the desired activity is obtained, one could repeat the experiment without the carrier to determine whether the carrier contributed to the activity.

The relevant quanta of permissible experimentation for a genetic claim cannot be the aggregate of work required to produce each and every embodiment potentially encompassed by the claims. If this were the case, it would never be possible to have a generic claim encompassing an unlimited number of species. Such claims have been upheld in numerous patents including those in unpredictable arts. *See, e.g., Ex parte Mark*, 12 USPQ2d 1904 (BPAI 1989); and, *In re Angstadt*, 190 USPQ 214 (CCPA 1976). The breadth of claims is relevant only insofar as the different species encompassed by the claims require different adaptations of an exemplified strategy. *See In re Strahilevitz*, 212 USPQ 561 (CCPA 1982) ("Although the invention is applicable to a large variety of haptens and antigens, the Examiner offered no reasons why these compounds would require *different techniques or process parameters*." *Ibid. at* p. 563 (emphasis supplied). Here, as discussed above, the application discloses a strategy of administering an agent and an adjuvant to generate antibodies against AB and thereby reducing or effecting prophylaxis of amyloid deposits. The application also provides examples of how agents can be screened for the desired activity using a transgenic mouse model. Other agents can be identified by routine repetition of the same procedure in the same transgenic model. Routine repetition of the same basic procedures to isolate additional agents operating according to the same principles to achieve the same results may require considerable experimentation but does not constitute undue experimentation.

¶28. Applicants request deferral of the obviousness-type double patenting issue until notification of otherwise allowable subject matter.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 650-326-2400.

Respectfully submitted,

Rosemarie L. Celli
Reg. No. 42,397

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 650-326-2400
Fax: 650-326-2422
RLC
60108972 v1

Down Syndrome and Alzheimer Disease, pages 123-142

DOWN SYNDROME AND ALZHEIMER DISEASE

B.T. Hyman
Neurology Service
Massachusetts General Hospital and Harvard Medical School
Fruit Street
Boston, MA 02114

Summary

The brains of individuals with Down's syndrome in their 40's and 50's begin to develop changes that are otherwise seen only in patients with Alzheimer disease. Neurons develop neurofibrillary tangles, flame-shaped alterations composed mainly of condensed cytoskeletal proteins. Another protein, β/A4 amyloid, is deposited in large amounts in the form of senile plaques and, around blood vessels, amyloid angiopathy. With increasing age, Down syndrome individuals accumulate more and more of these changes.

Different parts of the brain are affected to varying degrees by these two alterations. Surprisingly, the pattern of accumulation of neurofibrillary tangles and senile plaques is characteristic, and follows a predictable pattern. We have characterized this pattern in the hippocampal formation in a group of Down individuals, ages 13-71. Certain specific neurons such as those in layer II of entorhinal cortex and the CA1/subiculum field of the hippocampus are exquisitely vulnerable to tangle formation, and are the first neurons to be affected. Perhaps 20-30 years pass as the disease process evolves from mild to severe pathological changes.

One hypothesis for why Down individuals would be predisposed to developing Alzheimer pathology is the observation that the gene that encodes the precursor of the amyloid protein is located on chromosome 21. An extra copy of this gene, such as occurs in Down syndrome, may lead to "overproduction" of amyloid, and ultimately to its accumulation as senile plaques. Experiments to test this hypothesis are now underway.

Introduction

Down syndrome, the clinical manifestation of trisomy of chromosome 21, is a complex of physical signs that include a spectrum of mental retardation, congenital heart disease, dermatoglyphic changes and an early predisposition to developing the neuropathological and clinical features of Alzherimer disease. It is a common illness, occurring in approximately 1/1000 live births. As Mann (1988) has pointed out, the life expectancy of an infant with Down syndrome was only 9 years in 1929, whereas now with more aggressive treatment of infectious disease as many as 70% of individuals with Down syndrome can expect to live beyond age 50. A individual with Down syndrome live longer, it has been recognized that with increasing age essentially all middle aged Down individuals develop neurofibrillary tangles and senile plaques, the neuropathological hallmarks of Alzheimer disease. This review will summarize what is known about the relationship of Alzheimer disease and Down syndrome.

Clinico-pathological correlations

Down syndrome and Alzheimer disease are closely interrelated. After age 35, essentially 100% of patients with Down syndrome develop the neuropathological changes of Alzheimer disease (Burger and Vogel, 1973; see Kemper, 1988 for review). By contrast, in the general population neurofibrillary tangles and senile plaques are extremely rare until the mid-50's, and even then occur only in small numbers (Arriagada et al.,

1991). Wisniewski and colleagues (1985) examined 100 brains of patients with Down syndrome who were 1 to 74 years of age. Neurofibrillary tangles and senile plaques were found in 49 of 49 patients above the age of 30, and in 7 of 49 below that age. Ropper and Williams (1980) found plaques and tangles in all 20 cases of Down syndrome, examined at ages 30-69 years. It is also well documented that middle-aged individuals with Down syndrome commonly develop cognitive impairment in a pattern similar to that of Alzheimer disease. For example, Wisniewski et al. (1985b) provided a clinicopathological description of seven patients with Down syndrome over the age of 40 with documented progressive cognitive impairment extending from 2.5 to 9 years before death. At autopsy, each of these patients was shown to have developed severe accumulation of neurofibrillary tangles and senile plaques. Lai and Williams (1989) prospectively evaluated 96 patients with Down syndrome and found an increasing prevalence of dementia with age. Functional decline was seen in 55% of patients between 50 and 59, and 75% of patients over 60. Brains from all 12 autopsied cases (average age 62) showed large numbers of plaques and tangles, in the same locations as persons with Alzheimer disease. A study of 50 adult Down patients with mild mental retardation confirmed these figures. The prevalence of dementia increased from 0 in the group age 20-29, to 33% in the age group 30-39 to 55% in the age group 40-52, and all demented patients had signs of brain atrophy on CT (Franceschi et al., 1990). In our own study (Hyman and Mann, 1991) of 20 Down individuals ages 13-71, we found mild to moderate Alzheimer neuropathological changes in individuals ages 31-50, and marked changes in all individuals over age 50. Cognitive decline had been noted in all patients over the age of 58 (Table 1). Thus, mild or moderate neuropathological changes did not manifest as overt loss of cognitive ability, implying a presymptomatic stage of Alzheimer-type dementia that lasts 20-30 years.

Table 1. Characteristics of Down syndrome individuals[1]

	Age	Clinical Status[2]	Neuropathology[3]
DS-1	13	ND	None
DS-2	31	ND	Mild
DS-3	37	ND	Mild
DS-4	38	ND	Mild
DS-5	40	ND	Mild
DS-6	41	UNK	Mild
DS-7	42	ND	Mild
DS-8	47	ND	Moderate
DS-9	49	ND	Mild
DS-10	50	PrD	Moderate
DS-11	52	UNK	Marked
DS-12	53	ND	Marked
DS-13	58	D	Marked
DS-14	59	D	Marked
DS-15	59	D	Marked
DS-16	60	D	Marked
DS-17	60	D	Marked
DS-18	64	D	Marked
DS-19	65	D	Marked
DS-20	71	D	Marked

[1]Adapted from Hyman and Mann, 1991.
[2]D, demented; ND, not demented; PrD, probable dementia; UNK, unknown.
[3]As judged by neurofibrillary tangle accumulation in the hippocampal formation.

Patterns of neurofibrillary tangle and senile plaque pathology in Alzheimer disease and Down syndrome

Neurofibrillary tangles. It has been known for many years that neurofibrillary tangles and senile plaques preferentially accumulate in some areas of the cortex, and other areas tend to be spared (Hirano and Zimmerman, 1962; Hyman et al., 1984; Saper, Wainer and German, 1987; Rogers and

Morrison, 1985; Arnold et al., 1991). Recently, information gained from the study of neural connections in experimental animals has been applied to the topography of Alzheimer changes, allowing a re-analysis of the patterns of pathology (Hyman et al., 1984; Van Hoesen and Damasio, 1987 for review). This has led to the conclusion that specific sets of neurons, particularly projection neurons in defined cytoarchitectural fields in the hippocampal formation and association cortices, consistently develop neurofibrillary tangles (Hyman et al., 1984; Rogers and Morrison, 1985; Pearson et al., 1985; Arnold et al., 1991). Other neurons (for example, those in primary sensory or motor cortices) are resistent to neurofibrillary tangle degeneration.

The population of vulnerable neurons is remarkably consistent from case to case. For example, neurons that seem to be primarily responsible for providing afferent and efferent information to the hippocampal formation are severely affected by neurofibrillary changes (Hyman et al., 1984;1986, 1990) (Figure 1). These include layers II and IV of entorhinal cortex, and the CA1/subicular zone of the hippocampus. Other fields, sometimes immediately adjacent, are relatively spared. The cytoarchitectural fields of the hippocampal formation are involved in a consistent hierarchical fashion. Our recent semiquantitative studies showed that the following order of involvement was obeyed in each of 22 cases of Alzheimer disease assessed : Entorhinal cortex (layer II) > CA1, Subiculum , EC layer IV > CA3, CA4 > dentate gyrus, presubiculum (Hyman et al., 1992). Is this also the case in Down's syndrome? Elderly Down syndrome individuals who have developed Alzheimer pathology clearly accumulate tangles in the same hierarchy and in the same set of vulnerable neurons (Ball and Nuttall, 1980; Mann et al., 1986; Hyman and Mann 1991) The pattern of tangles in young Down patients has also been evaluated from this perspective, and these same areas of entorhinal cortex and hippocampus

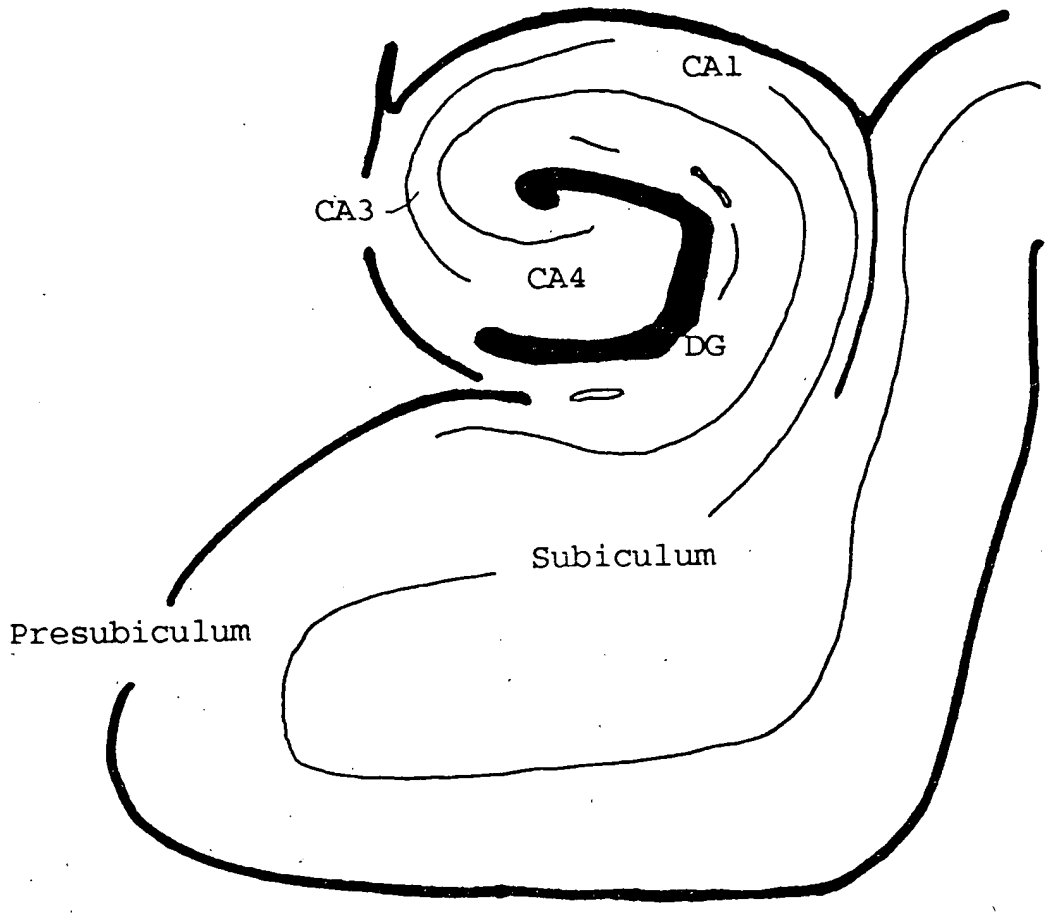

Figure 1. Anatomy of the human hippocampal formation. The majority of cortical input to the hippocampus is received by neurons in the entorhinal cortex. From here, the perforant pathway projects to the dentate gyrus (DG). A series of sequential intrinsic projections initiates from DG to CA3 (mossy fibers), then CA3 to CA1 (Schaeffer collaterals), then CA1 to subiculum (ammonic-subicular fibers). Primary output to cortex and limbic structures arises from areas CA1 and subiculum (see Hyman et al., 1990 for review).

appear to be vulnerable (Mann and Esiri, 1988; Hyman and Mann, 1991).

Significant advances have been made in the last five years in the biochemistry and molecular biology of neurofibrillary tangles. The primary identified component of tangles appears to be the microtubule associated protein, tau (Grundke-Iqbal et al., 1986; Wood et al., 1986 Kosik et al., 1986;1989; Nukina and Ihara, 1986 ; Wischik et al., 1988; Kondo et al., 1988; See Selkoe 1989 for review). Tau is a normal cytoskeletal protein that in the mature brain is located only in an axonal distribution. Its normal function appears to be to play a role in maintenance of neurite polarity (Caceres and Kosik,1990). In Alzheimer disease and in Down syndrome (Joachim, 1987; Flament et al., 1990), tau immunoreactivity appears in the somatodendritic compartment in association with neurofibrillary tangles. Neurons that contain neurofibrillary tangles remain capable of synthesizing tau, and <u>in situ</u> hybridization studies have shown that the mRNA for tau is present in neuronal soma and proximal dendrites (Kosik et al., 1989; Hyman et al., 1992)).

<u>Senile plaques.</u> A similar topographic hierarchical distribution can be mapped for senile plaques (Hyman et al., 1986;1992). In the Alzheimer hippocampus a discrete hierarchical distribution is found with CA1, Subiculum > dentate gyrus (molecular layer), entorhinal cortex layer III > CA4, CA3, presubiculum . Moreover, our results suggests that frequently senile plaques and A4 amyloid deposition are found in the terminal zones of degenerating neurons (Hyman et al., 1986; 1990). Immunocytochemical evidence from Dr. Masters' laboratory (Davies et al., 1988) suggests that A4 amyloid protein is deposited in the brain of individuals in the normal population over the age of 45, and increases in amount over the next one to two decades By age 80, over 80% of the population had A4 deposition. Thus it appears that, in the normal population, A4

deposition is one of the earliest manifestations of aging or of Alzheimer disease. Of importance to understanding the development of Alzheimer changes in Down syndrome, the same group has reported a similar pattern occurring in Down syndrome, but the age when A4 deposition begins is three decades earlier, in the teenage years (Masters and Beyreuther, 1988; Mann et al., 1990).

One major biochemical component of senile plaques is the β-A4 amyloid protein, first isolated by Glenner and Wong, 1984, and Masters et al. 1985. This 40 or 42 amino acid hydrophobic peptide is derived from amyloid precursor proteins (APP),. a family of alternatively spliced proteins of 563,695,751,or 770 amino acids See Tanzi, et al., 1989 for review). The 563, 751,and 770 forms all contain an insert of amino acids homologous to the Kunitz class of protease inhibitors, and so may have a different biological function than that of the 695 form (Tanzi et al., 1988). In situ hybridization studies using autoradiography (Bahmanyar et al., 1987; Lewis et al. 1987) have shown that neurons contain mRNA for APP, but that there is no clear relationship between the distribution of neurons that are at risk for tangle formation and those that have APP message. We have recently shown by both in situ hybridization and immunocytochemistry that both KPI containing and the 695 forms are present in neurons, and both contribute to the dystrophic neurites present surrounding some senile plaques (Hyman et al., 1992; Tanzi and Hyman, 1992). Neurons that contain tangles appear to also show APP mRNA (all 4 transcripts) and apparently continue to synthesize APP.

Initial Alzheimer changes in Down syndrome. Neuropathologic examination of young individuals with Down syndrome who have died before the fifth decade show a few tangles and plaques. According to the idea of hierarchical vulnerability, we predicted that these first neurofibrillary

tangles would occur in neurons in layer II of entorhinal cortex and the CA1/subicular field.

Preliminary evidence suggests that it is exactly this population of neurons that tends to develop neurofibrillary tangles at an early age in Down syndrome (Mann and Esiri,1988; Hyman and Mann, 1991). Plaques tend to accumulate in the CA1 and subicular fields, and in the molecular layer of the dentate gyrus (Hyman and Mann, unpublished).

One clue to the early changes that a cell undergoes before neurofibrillary tangle degeneration is provided by an immunocytochemical tool, Alz-50. This is a monoclonal antibody raised against an Alzheimer brain homogenate and selected for the ability to recognize Alzheimer, but not control, brains (Wolozin et al., 1986; Hyman, 1988). The antibody recognizes both A68, a 68kD soluble protein (Wolozin et al., 1986), which is likely an epitope of the tau molecule (Lee et al., 1991). The antigen is also present in Down brains from the second decade onward (Wolozin, 1988; Mattiace, 1989). Our studies in Alzheimer disease (Hyman et al, 1988) and in Down syndrome (Hyman and Mann, 1991) support the idea that neurons at risk to develop neurofibrillary tangles become immunoreactive for Alz-50.

Alz-50 and anti-tau immunocytochemistry are useful for visualizing neurofibrillary tangles, dystrophic neurites, and some neurons that are believed to be "pre-neurofibrillary tangles". Combining the power of the hierarchical vulnerability scheme we have developed in our studies of Alzheimer disease in which we can rank order cytoarchitectural fields in terms of involvement, and the predictive power of progression of Alzheimer pathology in the Down individuals, the hypothesis that Alz-50 and anti-tau recognize neurons that are destined to develop neurofibrillary pathology can be tested. As noted above, our initial studies suggest that Alz-50 does recognize "at-risk" neurons in Down syndrome (Hyman and Mann, 1991). These "at risk" neurons

show a diffuse stain over the cytoplasm which extends into the dendritic arborizations and the axon, giving an almost Golgi-like appearance (see, for example, Hyman et al., 1988). The immunohistochemical staining is not condensed as one sees with a neurofibrillary tangles, and the "at risk". Neurons can be shown not to contain a tangle by subsequent histological staining. Nonetheless, this is an abnormal staining pattern insofar as neither Alz-50 nor anti-tau immunocytochemistry reveals any staining in the somatodendritic compartment under normal circumstances. Alz-50 positive cells in the hippocampus have been reported to have about 30% less mRNA than Alz-50 negative cells, again suggesting that Alz-50 marks dysfunctional neurons (Doebler et al., 1988).

It is unknown whether amyloid deposition precedes, accompanies, follows, or is correlated to tangle formation in Alzheimer disease. Data from Dr. Mann's laboratory suggests that amyloid deposition precedes tangles (Mann and Esiri 1989), but other groups have reported the opposite (Bigio, et al., 1990). Our own data suggest that this is dependent on where you look: in the hippocampal formation and entorhinal cortex, neurofibrillary tangles may precede senile plaques, whereas the opposite is probably true in most cortical areas.

Molecular biological studies of Down syndrome and Alzheimer disease

Why does trisomy 21 lead to early development of Alzheimer disease? The most likely explanation is that the amyloid precursor protein gene resides on chromosome 21 (Robakis et al., 1989; Tanzi et al., 1987a; Goldgaber et al., 1987). Insight into the mechanism of why this is important has come from the genetic study of familial Alzheimer disease. Alzheimer disease is itself inherited in some families, independent of Down syndrome, and although there is clearly heterogeneity the gene

defect in some families lies on chromosome 21 (St. George-Hyslop et al., 1990). Although the great majority of these families do not demonstrate tight genetic linkage with the amyloid precursor protein gene (Tanzi et al., 1987b; Tanzi et al., in preparation), a recent exciting breakthrough has been the discovery that some Alzheimer families do have a mutation in the amyloid precursor protein that co-segregates with the disease (Goate, et al., 1991). This suggests that a defect in the amyloid precursor protein alone is sufficient to cause Alzheimer disease.

More direct proof of this idea has emerged in the last year from studies of transgenic mice that have been genetically engineered to "overproduce" amyloid precursor protein (or a fragment thereof). Cordell and colleagues (1991) showed doposition of β/A4 amyloid in animals that had extra copies of the Kunitz-protease containing amyloid precursor protein gene. More dramatic Alzheimer-like pathology, including neurofibrillary tangle like structures, was reported by Kawabata, Higgins and Gordon (1991) in animals transgenic for a fragment of the amyloid precursor protein. Neurofibrillary tangle-like structures were also reported in rats whose brains has been injected with β/A4 amyloid protein itself (Kowall et al., 1991).

Concluding remarks

These data suggest that individuals with Down syndrome will develop neurofibrillary tangles, and senile plaques in their 40's, and that the continued accumulation results in cognitive decline by the 50's or 60's. These occur in the same locations, and are the same biochemically and immunohistochemically, as the neurofibrillary tangles and senile plaques that characterize Alzheimer disease.

Experiments reported within the past year provide several lines of evidence that the crucial

component of the illness that predisposes Down patients to early Alzheimer-type pathology is the implication of the amyloid precursor protein gene on chromosome 21. This leads to accumulation of β amyloid and presumably, in a way not yet understood, to the development of neurofibrillary tangles in vulnerable populations of neurons. This leads to destruction of neural systems, and to ultimately cognitive impairment after compensatory mechanisms fail. Thus, treatment to prevent the development of Alzheimer disease in Down patients (and perhaps in the general population as well) will be aimed at reducing the expression and/or neurotoxic effects of β-amyloid.

Acknowledgements

I thank Dr. David Mann, Manchester England with whom some of the experiments described were performed, Ms.Kristin Marzloff for expert technical assistance, and Ms. Sharon Melanson for organizing the manuscript. Supported by an Alzheimer's Association Faculty Scholar Award, NIH AG08487, and a NATO Fellowship.

References

Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW (1991): Topographic distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex in Alzheimer's disease. Cerebral Cortex 1:103-116.

Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT (1992): Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer disease. Neurology, in press.

Bahmanyar S, Higgins GA, Goldgaber D, Lewis DA, Morrison JH, Wilson MC, Shankar SK, Gajdusek DC (1987): Localization of amyloid b protein messenger RNA in brains from patients with Alzheimer disease. Science 237:77-80

Ball MJ, Nuttall K (1980): Neurofibrillary tangles and granulovacuolar degeneration and neurone loss in Down's syndrome: quantitative comparison with Alzheimer dementia. Ann Neurol 7:462-465.

Bigio EH, Sparkman DR, Clark AW, White CL III (1990): Neurofibrillary tangles precede amyloid plaques in Down syndrome. J Neuropath Exp Neurol 49:265.

Burger PC, Vogel FS (1973): the development of the pathologic changes of Alzheimer's disease and senile dementia in patients with Down's syndrome. Am J Path 73:457-476.

Caceres A, Kosik KS (1990): Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. Nature 343:461-463.

Davies L, Wolska B, Hilbich C, Multhaup G, Martins R, Simms G, Beyreuther K, Masters CL (1988): A4 amyloid protein deposition and the diagnosis of Alzheimer's disease: Prevalence in aged brains determined by immunocytochemistry compared with conventional neuropathologic techniques. Neurology 38:1688-16993.

Doebler JA, Markesbery WR, Anthony A, Davies P, Scheff SW, Rhoads RE (1988): Neuronal RNA in relation to ALZ-50 immunoreactivity in Alzheimer's disease. Ann Neurol 23:20-24.

Flament S, Delacourte A, Mann DMA (1990): Phosphorylation of tau proteins: a major event during the process of neurofibrillary degeneration. A comparative study between Alzheimer's disease and Down's syndrome. Brain Res 516:15-19.

Franceschi M, Comola M, Piattoni F, Gualandri W, Canal N (1990): Prevalence of dementia in adult patients with trisomy 21. Am J Med Genet 7:306-308.

Glenner GG, Wong CW (1984): Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Comm 122:1131-1135.

Goate AM, Chartier-Harlin MC, Mullan MC, Brown J, Crawford F, Fidani L, Guiffra A, Haynes A, Irving N, James L, et al. (19): Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349:704-706.

Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC (1987): Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 235:877-880.

Grundke-Iqbal I, Iqbal K, Tung Y-C, et al. (1986): Abnormal phosphorylation of the microtubule-associated protein t (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci USA 83:4913-4917.

Hirano Z, Zimmerman HM (1962): Alzheimer's neurofibrillary changes. A topographic study. Arch Neurol 7:73-88.

Hyman BT, Mann DMA (1991): Alzheimer type pathological changes in Down's syndrome individuals of various ages. In Iqbal K, McLachlan DRC, Winblad B, Wisniewski HM (eds): "Alzheimer's Disease: Basic Mechanisms, Diagnosis, and Therapeutic Strategies." New York: John Wiley and Sons,pp 105-113

Hyman BT, Damasio AR, Van Hoesen GW, Barnes CL, (1984): Cell specific pathology isolates the hippocampal formation in Alzheimer's disease. Science 225:1168-1170.

Hyman BT, Van Hoesen GW, Kromer LJ, Damasio AR (1986): Perforant pathway changes and the memory impairment of Alzheimer's disease. Ann Neurol 20:473-482.

Hyman BT, Van Hoesen GW, Wolozin B, Davies P, Damasio AR (1988): Alz-50 antibody recognizes Alzheimer-related neuronal changes. Ann Neurol 23:371-379.

Hyman BT, Van Hoesen GW, Damasio AR (1990): Memory-related neural systems in Alzheimer's disease: An anatomical study. Neurology 40:1721-1730.

Hyman BT, Tanzi RE, Marzloff KM, Barbour R, Schenk D (1992): Kunitz protease inhibitor containing amyloid precursor protein immunoreactivity in Alzheimer's disease: A quantitative study. J Neuropath Exp Neurol, in press.

Hyman BT, Kosik K, Tanzi RE (1992): In situ hybridization of mRNA for tau, MAP-1b and MAP-2 in the hippocampal formation in Alzheimer's disease. Neurology (abstract), in press.

Hyman BT, Arriagada PV, Van Hoesen GW, Damasio AR (1992): Memory impairment in Alzheimer's disease: An anatomical perspective. In Parks RW, Zec RF, Wilson RS (eds): "Neuropsychology of Alzheimer's Disease and other Dementias." New York: Oxford Press, in press.

Joachim CL, Morris JH, Selkoe DJ (1988): Clinically diagnosed Alzheimer's disease autopsy results in 150 cases. Ann Neurol 24:50-56.

Kemper TL (1988): Neuropathology of Down syndrome. In Nadel L (ed): "The Psychobiology of Down Syndrome." Cambridge, MA: MIT Press, pp 269-289.

Kawabata S, Higgins GA, Gordon JW (1991): Amyloid plaques, neurofibrillary tangles and neuronal loss in brains of transgenic mice overexpressing a C-terminal fragment of human amyloid precursor protein. Nature 354:476-478.

Kosik KS, Joachim CL, Selkoe DJ (1986): Microtubule associated protein tau (t) is a major antigenic determinant of PHF in Alzheimer's disease Proc Natl Acad Sci USA 83:4044-4048.

Kosik KS, Crandall JE, Mufson EJ, Neve RL (1989): Tau in situ hybridization in normal and Alzheimer brain: Localization in the somatodendritic compartment. Ann Neurol 26:352-361.

Kowall NW, Beal MF, Busciglio J, Duffy LK, Yankner BA (1991): An in vivo model for the neurodegenerative effects of b amyloid and protection by substance P. Proc Natl Acad Sci USA 88:7247-7251.

Lai F, Williams RS (1989): A prospective study of Alzheimer disease in Down syndrome. Arch Neurol 46:849-853.

Lee VMY, Balin B, Otvos L, Trojanowski JQ (1991): A68: A major subunit of paired helical filaments and derivatized forms of normal tau. Science 251:675-678.

Lewis DA, Campbell MJ, Terry RD, Morrison JH (1987): Laminar and regional distributions of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: A quantitative study of

visual and auditory cortices. J Neurosci 7:1799-1808.

Mann DMA, Yates PO, Marcyniuk B, Ravindra CR (1986): The topography of plaques and tangles in Down's syndrome patients of different ages. Neuropathol Appl Neurobiol 12:447-457.

Mann DMA (1988): The pathological association between Down syndrome and Alzheimer disease. Mech Ageing Develop 43:99-136.

Mann DMA, Esiri MM (1988): The site of the earliest lesions of Alzheimer's disease. N Engl J Med 318:789-790.

Mann DMA, Jones D, Prinja D, Purkiss MS (1990): The prevalence of amyloid (A4) protein deposits within the cerebral cerebellar cortex in Down's syndrome and Alzheimer's disease. Acta Neuropathol 80:318-327.

Masters CL, Beyreuther K (1988): The molecular pathology of the amyloid A4 precursor of Alzheimer's disease. Ann Mtg Scandinavian Neuropath Soc, #24.

Masters CL, Simms G, Weinmann NA, Multhaup G, McDonald BL, Beyreuther K (1985): Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. Proc Natl Acad Sci USA 82:4245-4249.

Mattiace LA, Dickson DW, Kress YS, Davies P (1989): ALZ-50 immunoreactivity precedes PHF formation and neuritic change in Down's syndrome. Soc. Neurosci. 15:1038.

Pearson RCA, Esiri MM, Hiorns RW, Wilcock GK, Powell TPS (1985): Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer's disease. Proc Natl Acad Sci USA 82:4531-4534.

Robakis NK, Ramakrishna N, Wolfe G, Wisniewski HM (1987): Molecular cloning and characterization

of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. Proc Natl Acad Sci USA 84:4190-4194.

Rogers J, Morrison JH (1985): Quantitative morphology and regional laminar distributions of senile plaques in Alzheimer's disease. J Neurosci 5:2801-2808.

Ropper AH, Williams RS (1980): Relationship between plaques and tangles and dementia in Down's syndrome. Neurology 30:639-644.

Saper CB, Wainer BH, German DC (1987): Axonal and transneuronal transport in the transmission of neurological disease: Potential role in system degenerations, including Alzheimer's disease. Neuroscience 23:389-398.

Selkoe DJ (1989): Biochemistry of altered brain proteins in Alzheimer's disease. Ann Rev Neurosci 12:463-490.

St. George-Hyslop PH, Haines JL, Farrer LA, et al. (1990): Genetic linkage studies suggest that Alzheimer's disease is not a single homogeneous disorder. Nature 347:194-200.

Tanzi RE, Hyman BT (1992): "Studies of amyloid b precursor expression in Alzheimer's disease." Wurtman R, Corkin S, Growdon JH (eds) Ann NY Acad Sci., in press.

Tanzi RE, Gusella JF, Watkins PC, Bruns GAP, St. George-Hyslop P, Van Keuren ML, Patterson D, Pagen S, Kurnit DM, Neve RL (1987): Amyloid b protein gene: cDNA, mRNA distribution and genetic linkage near the Alzheimer locus. Science 235:880-884.

Tanzi RE, St. George-Hyslop PH, Haines JL, Polinsky RJ, Nee L, Foncin J-F, Neve RL, McClatchey AI, Conneally PM, Gusella JF (1987): The genetic defect in familial Alzheimer's

disease is not tightly linked to the amyloid protein gene. Nature 329:156-157.

Tanzi RE, McClatchey AI, Lamperti ED, et al. (1988): Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. Nature 331:528-530.

Tanzi RE, St. George-Hyslop PH, Gusella JF (1989): Molecular genetic approaches to Alzheimer's disease. TINS 12:152-158.

Van Hoesen GW, Damasio AR (1987): Neural correlates of cognitive impairment in Alzheimer's disease. In Mountcastle VB, Plum F, Geiger SR (eds): "The Nervous System." Baltimore: The William & Wilkins Company, pp 871-898

Wischik CM, Novak M, Thogersen HC, Edwards PC, Runswick MJ, Jakes R, Walker JE, Milstein C, Roth M, Klug A (1988): 3.Isolation of a Fragment of Tau Derived from the Core of the Alzheimer Paired Helical Filament. Proc Natl Acad Sci USA 85:4506-4888.

Wisniewski KE, Wisniewski HM, Wen GY (1985): Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. Ann Neurol 17:278-282.

Wisniewski KE, Dalton AJ, Crapper-McLachlan DR, Wen GY, Wisniewski HM (1985): Alzheimer's disease in Down's syndrome. Neurology 35:957-961.

Wolozin BL, Pruchnicki A, Dickson DW, Davies P (1986): A neuronal antigen in the brains of Alzheimer patients. Science 232:648-650.

Wolozin B, Scicutella A, Davies P (1988): Reexpression of a developmentally regulated antigen in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 85:6202-6206.

Wood JB, Mirra SSS, Pollock NJ, Binder LI (1986): Neurofibrillary tangles of Alzheimer's disease share antigenic determinants with the axonal microtubule-associated protein tau. Proc Natl Acad Sci USA 83:4040-4043.

Exp Neurol 1992;118:117-125.
35. Pouplard-Barthelaix A. Immunological markers and neuropathological lesions in Alzheimer's disease. In: Pouplard-Barthelaix A, Emile J, Christen Y, eds. Immunology and Alzheimer's disease. Berlin: Springer, 1988:7-16.
36. Johnson SA, Lampert-Etchells M, Pasinetti GM, Rozovsky I, Finch CE. Complement mRNA in the mammalian brain: responses to Alzheimer's disease and experimental brain lesioning. Neurobiol Aging 1992;13:641-648.
37. Rogers J, Cooper NR, Webster S, et al. Complement activation by B-amyloid in Alzheimer disease. Proc Natl Acad Sci USA 1992;89:10016-10020.
38. McGeer PL, Rogers J. Anti-inflammatory agents as a therapeutic approach to Alzheimer's disease. Neurology 1992;42:447-449.
39. Rogers J, Kirby LC, Hempelman SR, et al. Clinical trial of indomethacin in Alzheimer's disease. Neurology 1993;43:1609-1611.

Cognitive impairment in adults with Down's syndrome:

Similarities to early cognitive changes in Alzheimer's disease

K.L. Brugge, MD; S.L. Nichols, PhD; D.P. Salmon, PhD; L.R. Hill, PhD; D.C. Delis, PhD;L. Aaron; and D.A. Trauner, MD

Article abstract—Postmortem studies of brains from adults with Down's syndrome (DS) reveal a dramatic age-dependent increase in the incidence of neuropathology associated with Alzheimer's disease (AD). By the age of 40 years, virtually all DS individuals have AD neuropathology. Documentation of cognitive correlates of this phenomenon has been difficult, partly because of the preexisting mental retardation in DS. In the current study, we compared a group of adults with DS, 22 to 51 years old, with a matched control group on various behavioral measures such as savings scores, which are known to be sensitive in detecting early dementia in AD patients. By using the short delayed savings score from the California Verbal Learning Test (a test of verbal memory), a subgroup of DS adults was identified as memory-impaired. This group demonstrated a decline in performance on various other cognitive tests with advancing age, whereas another group identified as having non–memory-impaired DS, and the non-DS controls, showed no evidence of decline with age. These results provide evidence for the presence of early dementia among adults with DS within an age range in which neuropathologic manifestations of AD are predicted to be developing.

NEUROLOGY 1994;44:232-238

Down's syndrome (DS) is a genetic disorder involving an excess of chromosome 21 (trisomy 21 in approximately 96% of the cases), and those with DS constitute approximately 15% of the population with mental retardation.[1] Several investigators reported a discrepancy between the presence of neuropathologic hallmarks[2] of Alzheimer's disease (AD) and dementia[3,4] in DS. Based on results of postmortem studies, almost all individuals with DS have neurofibrillary tangles and neuritic plaques (changes associated with AD in nonretarded individuals) present in their brains by age 40 years. However, previous studies[3-10] identified dementia among only some DS subjects over 40 years old. In many of these studies, DS subjects defined as demented were compared with a group of DS subjects of similar age who may also have been exhibiting early changes of dementia not detected by the measures employed. Furthermore, the presence of preexisting mental retardation in most individuals with DS presents a challenge in the detection of dementia in this population.

The potential cognitive changes associated with dementia of the Alzheimer type (DAT) in DS are not well characterized nor have sensitive markers detecting early dementia been identified. Measures of memory function, particularly delayed recall or

From the Departments of Neurosciences (Drs. Brugge, Nichols, Salmon, Hill, and Trauner, and L. Aaron) and Psychiatry (Dr. Delis), University of California, San Diego, School of Medicine, La Jolla, CA.

Supported by NIA Physician Scientist Award AG00353-05; by the NINDS Multidisciplinary Research Center for the Study of the Neurological Basis of Language, Learning and Behavior Disorders in Children (NS 22343); and in part by the General Clinical Research Center Grant M01 RR00827.

Received May 22, 1993. Accepted for publication in final form July 28, 1993.

Address correspondence and reprint requests to Dr. Karen Brugge, Department of Psychiatry, Box 1007, New England Medical Center, 750 Washington Street, Boston, MA 02111.

measures of the percent of memory retention over time (referred to as "savings scores") are sensitive and fairly specific in the detection of early DAT among the non-DS population.[11-15] Welsh et al[13,14] reported the savings score from the CERAD neuropsychological test battery to be the second best variable in discriminating between AD patients with *mild* DAT and nondemented age-matched controls, using various tests of memory and language (ie, the Boston Naming Test [BNT] and Category Fluency) and a test of constructional praxis. Delayed recall was the best detector of mild DAT in the study of Welsh et al. However, these investigators excluded from the statistical analysis data from subjects who recalled fewer than two items on short delay. Other investigators,[12] who did not employ this exclusionary criterion and obtained savings scores using the Logical Memory and Visual Reproduction subtests of the Wechsler Memory Scale-Revised (WMS-R), reported the savings score as the most sensitive and specific marker for early DAT. Intrusion errors or false positives that reflect other subtle aspects of memory function are reported to occur in the later stages of DAT.[13-15]

Children[16] or adults with DS,[17] when compared with control subjects of similar age and overall intelligence, show greater impairment in specific verbal tasks. Such impairment in nonmemory verbal performance may precede the onset of dementia in DS; therefore, nonmemory language tests such as Category Fluency and the BNT, which reveal impairments particularly in the *advanced* stages of DAT in AD patients,[14] may not be sensitive in detecting *early* dementia among DS adults.

The purpose of the present study was to determine whether cognitive impairments associated with early dementia in DS adults could be detected by measures that are sensitive in detecting dementia in AD patients. Since the DS subjects of the present study were within the age range in which the age-dependent increase of AD neuropathology in DS is known to be the greatest,[2] an age-dependent decline of memory function reflected by the savings score or delayed recall is predicted to exist among the DS but not the control subjects.

Methods. *Subjects.* Seventeen subjects with DS and seven age-matched, mentally retarded controls participated in the study. All subjects resided in two group homes in southern California. The mean age of the DS group, 31.1 ± 2.9 years (range, 22 to 51), was not significantly different from that of the seven controls, 28.9 ± 2.8 years (range, 22 to 46). The control subjects were also not significantly different from the DS group in mean Full Scale Intelligence Quotient (60.9 ± 2.8 for controls and 55.3 ± 1.6 for DS subjects), Performance IQ (controls, 64.9 ± 1.2; DS, 61.4 ± 1.5), or Verbal IQ (controls, 60.9 ± 2.9; DS, 55.5 ± 1.4) of the Wechsler Adult Intelligence Scale-Revised (WAIS-R).[18] All subjects had a complete physical evaluation, including neurologic and psychiatric examinations, and medical histories were obtained. None of the subjects had active major medical disorders (such as heart disease, infections, lung disease, liver disease, kidney disease, or diabetes) other than the neurologic condition resulting in moderate mental retardation. The diagnoses of the mentally retarded controls included hydrocephalus with a normal-pressure shunt, translocation of chromosomes 5/X or chromosomes 19/20, microcephaly, cerebral palsy, and unknown etiologies of mental retardation. All were ambulatory and active in daytime workshops or employment programs. Routine laboratory blood tests on each subject included CBC, BUN, creatinine, glucose, platelets, and chemistry profile and were within normal limits in all subjects. Since DS is associated with a high incidence of hypothyroidism, blood thyroxine and T_3RU levels were obtained. Although four DS subjects were taking thyroxine; all subjects showed thyroxine and T_3RU levels within the normal range. One DS subject and four controls were taking anticonvulsant medication. However, each of these had had their most recent seizure more than 1 month prior to the time of study, and most of them had had their last seizure several years prior to the study. Four of the seven control subjects were receiving a low dose of psychotropic medication such as 1 mg of haloperidol (orally) daily or a tricyclic antidepressant, and three were on medroxyprogesterone or primidone. Five DS subjects and three controls were taking daily vitamins, typically multivitamins. Subjects taking these various medications performed within two standard deviations of their respective group means on all neuropsychological test variables. All subjects were nonsmokers and on a similar diet, provided in the group home. None was obese.

Neuropsychological testing. All subjects were administered the following battery of neuropsychological tests: WAIS-R,[18] Logical Memory and Visual Reproduction subtests of the WMS-R,[19] California Verbal Learning Test-children's version (CVLT-C),[20] BNT,[21] Peabody Picture Vocabulary Test-Revised (PPVT-R),[22] Memory for Sentences (Memsent) and Memory for Objects from the Stanford-Binet Intelligence Scale, fourth edition,[23] Letter (Controlled Oral Word Association Test) and Category (Animals) Fluency tests,[24] Beery Developmental Test of Visual-Motor Integration,[25] Opposites subtest of the McCarthy Scales of Children's Abilities,[26] and Finger Tapping for the dominant (Tap Dom) and nondominant (Tap NonDom) hands from the Halstead-Reitan Battery.[27] The difference between the dominant and nondominant hand on Finger Tapping (DifferTap) was also obtained. The number of intrusion errors during generation of words starting with the letters "F," "A," or "S" on the Letter Fluency test was recorded and referred to as "FAS intrusions." The data on some subtests from one of the seven controls and one DS subject were missing or excluded due to the subject's unwillingness to respond.

The Logical Memory subtest from the WMS-R assessed immediate (I) and delayed (30-minute delay; II) recall of two paragraphs, A and B (Logical Memory IA and IB and Logical Memory IIA and IIB). A savings score for Logical Memory (expressed as percentage) was obtained with the following formula: Logical Memory IIA + IIB/Logical Memory IA + IB multiplied by 100. The Visual Reproduction subtest assessed immediate (VRIAD) and delayed (30-minute delay; VRIIAD) recall of four designs (designs A through D). A savings score for Visual Reproduction (expressed as percentage) was obtained by calculating the following: VRIIAD/VRIAD × 100.

The children's version of the CVLT was chosen to avoid the floor effect that might be obtained with the adult version. Since this test is not widely used, we provide a description. The CVLT-C is a verbal list-learning

task that provides measures of several aspects of learning and memory. On each of five trials, 15 words (list A) were presented orally at the rate of one word per second, and immediate free recall of the words was elicited. A second list (list B) was presented for one trial immediately after trial 5. Following the list B trial, the subject was asked to again recall the items of list A (short delayed free recall) and then to recall the words from list A with semantic cues provided (short delayed cued recall). After a 20-minute interval during which unrelated nonverbal tests were administered, the subjects were given free recall and category-cued recall trials (long delayed free recall and long delayed cued recall, respectively) and a recognition test of list A. The yes/no recognition test consisted of 30 distractor items intermixed with the 15 items of list A. The number of false-positive responses on the recognition test and the number of intrusions over all test trials were recorded. Savings scores for short and long delayed recall (expressed as percentages) were obtained by dividing short delayed free or long delayed free recall, respectively, by trial 5 recall multiplied by 100.

Statistical analysis. A stepwise logistic regression analysis comparing the DS and control subjects was performed with measures known to be sensitive to cognitive impairment in the early stages of Alzheimer's disease (hereafter referred to as "Alzheimer-related variables"). Stepwise logistic regression serves the same purpose as stepwise discriminant analysis in that it uses explanatory variables to determine to which of two groups (DS or control) each individual in the population belongs. The major difference is that, unlike discriminant analysis, logistic regression does not assume that the explanatory variables are normally distributed within each group. Since this assumption was obviously violated for several of the variables we were considering, we chose logistic regression as the better technique to use. The variables included in this analysis were Logical Memory IIA and IIB; VRIAD, VRIIAD, and the Visual Reproduction savings score from the WMS-R; false positives; intrusions on short cued recall and long cued recall; short and long delayed savings scores and trials 1-5 recall from the CVLT-C; FAS intrusions from the Letter Fluency test; and the score from the BNT. The Logical Memory savings score was not included in this analysis since several subjects received a score of zero on the Logical Memory immediate recall condition. Short and long free recalls of the CVLT-C were not included among the Alzheimer-related variables due to a floor effect of these variables among the DS subjects (raw mean scores of the DS subjects were 34 ± 1.0 and 4.2 ± 1.2; the raw mean scores of the control subjects were 6.2 ± 0.8 and 5.0 ± 1.4).

The stepwise logistic regression model was designed to determine which of the above variables best discriminates between DS and control subjects. The procedure considered all the Alzheimer-related variables and entered the best discriminating variable first in the model. Subsequently, the model entered the second variable with the greatest additional discriminating power (while considering all the Alzheimer-related variables) in the presence of the first variable. Due to an insufficient number of subjects, the third best discriminating variable could not be entered in the model. Since this analysis could not provide reliable group differences beyond the two best discriminating variables, separate t tests were performed to compare the performance of the DS and control groups on each of the Alzheimer-related variables. Statistical analysis on all psychometric variables in the present study was performed on raw scores. Since the DS subjects were predicted to show a greater impairment of performance compared with controls, the level of significance for a one-tailed test is reported.

Results. *Comparisons of the DS and control groups on Alzheimer-related variables.* Despite equivalent levels of general intellectual ability, DS subjects were significantly more impaired than the mentally retarded control subjects on the following Alzheimer-related variables: short delayed savings score ($p < 0.001$), long delayed savings score ($p < 0.01$), false positives ($p < 0.025$), BNT ($p < 0.025$), VRIAD ($p < 0.05$), and intrusion short and long cued ($p < 0.05$ for both measures). Using the Bonferroni correction (a criterion of $p < 0.001$ to be significant), the short delayed savings score was the only Alzheimer-related variable to show a significantly greater impairment in the DS group. With the Bonferroni correction, other variables showed trends for greater impairment in DS compared with controls. Short and long free recall failed to show significant differences between the DS and control groups due to floor effects. The DS group also showed significantly greater impairment than did controls on the following psychometric variables not included among the Alzheimer-related variables: DifferTap, PPVT, WAIS-R similarities, and Memsent (ranging from $p < 0.05$ to 0.001). (The mean raw diagnostic group scores and the results of the t test comparisons without Bonferroni correction between the DS and control groups on the Alzheimer-related variables and on other psychometric variables are in table 1, filed with the National Auxiliary Publications Service [NAPS]; see Note at end of article.)

A stepwise logistic regression analysis on Alzheimer-related variables revealed that the short delayed savings score from the CVLT-C was the variable that best discriminated between DS subjects and controls ($p < 0.005$, $\chi^2 = 9.30$), in that it was the only variable to show a group difference sufficient to enter the model. Among all psychometric variables, the short delayed savings score was the only variable to be significantly ($r = -0.544$, $p < 0.02$) inversely correlated with age in the DS group but not in the controls (see table 2, filed with NAPS). The control group showed either no correlation or significant positive correlations of Alzheimer-related variables with age (Logical Memory IIA, $r = 0.847$, $p < 0.05$, and Logical Memory IIB, $r = 0.822$, $p < 0.025$). Furthermore, unlike the DS group, the control group showed a significant positive correlation (ranging from an $r = 0.691$, $p < 0.05$, to $r = 0.862$, $p < 0.01$) of Logical Memory IA and IB subtests and WAIS-R Comprehension, Information, and Vocabulary subtests, and a trend for a positive correlation of the Picture Completion subtest ($r = 0.640$) with age (see table 2, filed with NAPS).

Segregation of DS subjects into memory-impaired and memory-nonimpaired groups. Performance on the short delayed savings score was chosen as the cognitive measure to theoretically classify the DS subjects into a demented ("memory-im-

paired") or nondemented ("memory-nonimpaired") category since this variable was found to be (1) the only age-dependent variable in the DS subjects among more than 25 dependent variables, (2) not age-dependent in controls, (3) the variable that best discriminated DS from controls based on stepwise logistic regression analysis, and (4) a sensitive cognitive marker for *early* dementia in AD patients.

The memory-impaired DS subjects were defined as those with a short delayed savings score of zero (no retention after a short delay), whereas the memory-nonimpaired DS subjects had short delayed savings scores of greater than zero (ranging from 67% to 167%). The range of this savings score among the controls was 27% to 100%. Since one DS subject failed to recall any items on trial 5, the data for this subject were excluded in subsequent analyses involving the two DS subgroups. A fairly even distribution of subjects was observed among the three diagnostic groups: controls (N = 7), memory-impaired (N = 7) and memory-nonimpaired DS (N = 8) groups. The term "memory-nonimpaired" is relative in this context in that the memory-nonimpaired DS and the control groups were not significantly different in their mean short delayed savings scores (77.4 ± 7.5 for the memory-nonimpaired DS group and 110.36 ± 14.6 for the control group).

The two DS subgroups did not differ in mean Full Scale IQ (54.3 ± 1.9 for the memory-impaired and 57.1 ± 2.9 for the nonimpaired group), Performance IQ (61.7 ± 1.8 for the memory-impaired and 62.3 ± 2.9 for the nonimpaired), or Verbal IQ (54.4 ± 1.7 for the memory-impaired and 57.1 ± 2.4 for the nonimpaired). However, the memory-impaired DS subjects were significantly ($p < 0.01$, $t = 3.016$, $df = 13$) older than the memory-nonimpaired DS subjects (mean ages, 38.6 ± 3.8 years for the memory-impaired and 27.9 ± 1.4 years for the nonimpaired). Although the control group had a mean age (28.9 ± 2.8 years) similar to that of the memory-nonimpaired group, it did not differ significantly from either DS subgroup.

Comparisons between memory-impaired and memory-nonimpaired DS groups and controls on various measures of cognitive performance. Comparisons (using ANOVA) among the three diagnostic groups on various psychometric measures revealed main effects among the three groups for most of those variables in which significant differences existed between the controls and the entire DS group (see table 1, filed with NAPS). The Alzheimer-related variables showing diagnostic group differences included the short delayed savings score ($p < 0.001$), long delayed savings score ($p < 0.01$), false positives ($p < 0.025$), and VRIAD ($p < 0.05$). Other Alzheimer-related variables, such as intrusions (FAS, and short and long cued) failed to show significant diagnostic group differences. In addition, a significant group effect was observed for three other measures of memory function, short free recall ($p < 0.001$), long free recall ($p < 0.05$), and Memsent ($p < 0.025$), and for a psychomotor measure, DifferTap ($p < 0.025$). BNT, PPVT, and the WAIS-R similarities subtest showed trends for diagnostic group effects ($p < 0.075$, $F = 2.94$).

Post hoc *t* tests were performed on those variables showing significant diagnostic group effects by ANOVA. Memory-nonimpaired DS subjects failed to differ significantly from the control subjects on almost all the variables. In contrast, the memory-impaired DS group was significantly worse than control subjects on the following variables: short and long delayed savings score ($p < 0.001$), short ($p < 0.001$) and long ($p < 0.05$) free recall, false positives ($p < 0.05$), VRIAD ($p < 0.05$), Memsent ($p < 0.01$), and DifferTap ($p < 0.01$) (table 1, filed with NAPS). The magnitude of impairment exhibited by the memory-impaired DS group tended to be greater than that of the memory-nonimpaired DS subjects on several variables. However, only the short ($p < 0.001$) and long ($p < 0.025$) delayed savings scores and short free recall ($p < 0.001$) showed significant differences between the two DS subgroups.

Correlation of neuropsychometric measures with age in memory-impaired DS, memory-nonimpaired DS, and controls. Since the DS group as a whole failed to show significant correlations with age on any variable while the controls showed positive correlations, it was hypothesized that the memory-impaired subgroup included in the larger DS cohort was masking age effects that might exist in the memory-nonimpaired DS. If this were the case, nonimpaired DS would be expected to show correlations similar to those observed in the controls (ie, improvement of WAIS-R verbal subtests with age) while memory-impaired DS would be expected to show no correlation or a decline in performance with age. Pearson product-moment correlations of several variables with age for the three diagnostic groups are shown in figures 1 through 3. (Pearson product-moment correlations for Alzheimer-related variables, WAIS-R subtests, and other psychometric variables are shown for all diagnostic groups in table 2, filed with NAPS.)

Segregation of the DS groups into memory-impaired and nonimpaired subgroups revealed that the nonimpaired DS subgroup showed significant positive correlations or trends for positive correlations between age and various WAIS-R verbal subtests (ranging from $r = 0.645$ to 0.735, $p < 0.05$ to 0.025) and tests of memory (Logical Memory IB, $r = 0.640$, $p < 0.05$), similar to those of the controls (ranging from $r = 0.691$ to 0.862, $p < 0.05$ to 0.01 for WAIS-R verbal subtests, and ranging from $r = 0.780$ to 0.861, $p < 0.05$ to 0.01 for all four Logical Memory subtests). In contrast, the memory-impaired DS group failed to show improvement in cognitive function with age on any of the variables. Instead, the memory-impaired DS subgroup showed a significant decline, or trend for a decline (ranging from $r = -0.668$ to -0.835, $p < 0.05$ to 0.01), in cognitive performance with advancing age on a number of measures. These measures included several Alzheimer-related variables, other measures of memory function (Logical Memory subtests, trial 1-5 recall, list B recall), and nonmemory variables including the

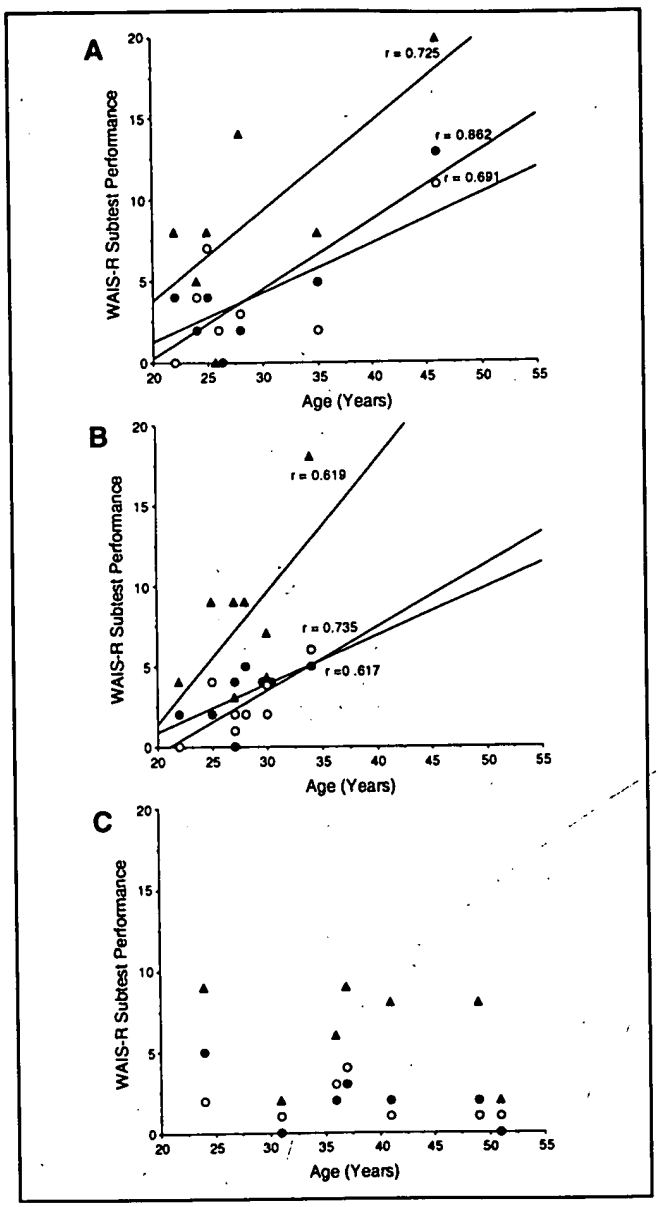

Figure 1. Correlations of age with performance on the WAIS-R subtests; Information (open circle), Vocabulary (triangles), and Comprehension (closed circles) for each diagnostic group: (A) controls, (B) memory-nonimpaired Down's, and (C) memory-impaired Down's.

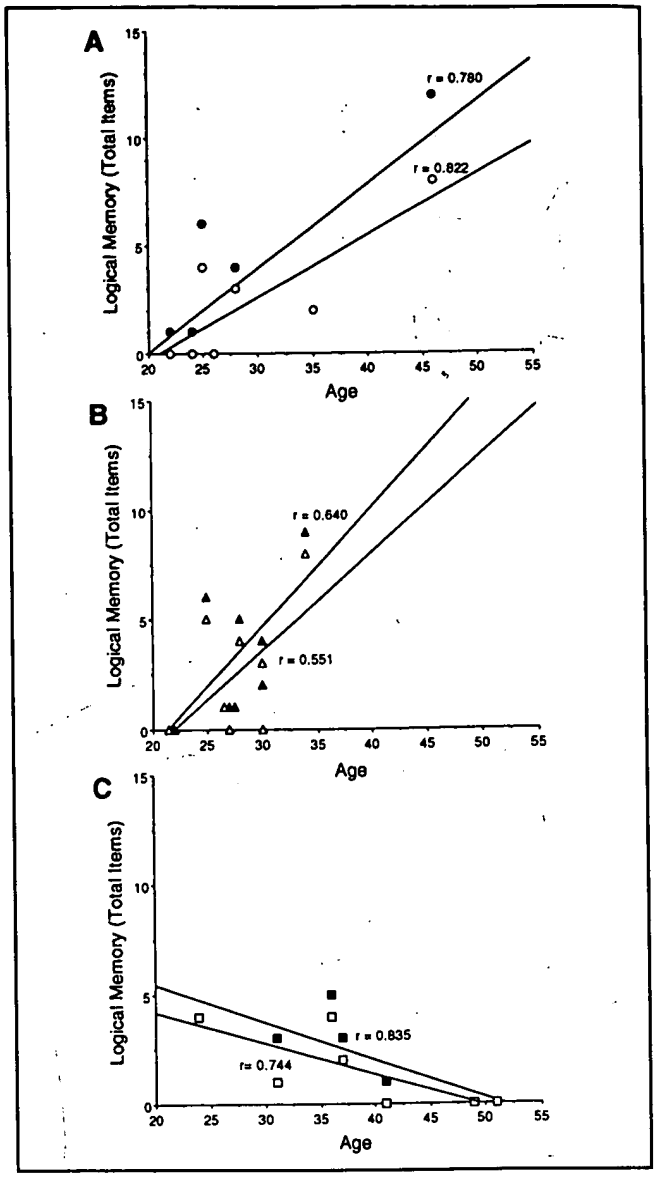

Figure 2. Correlations of age with immediate Logical Memory recall (closed circles) and delayed Logical Memory recall (open circles) of part B of the Wechsler Memory Scale-Revised for each diagnostic group: (A) controls, (B) memory-nonimpaired Down's, and (C) memory-impaired Down's.

WAIS-R Block Design subtest and Finger Tapping (for Tap NonDom and Tap Dom, see figure 3; see also table 2, filed with NAPS).

Discussion. In the present study, the short delayed savings score (obtained from the CVLT-C), a sensitive cognitive marker for early DAT in AD patients,[12-15] (1) declined with advancing age in the DS group but not in the controls, and (2) discriminated DS from controls based on stepwise logistic regression analysis. Other psychometric measures, which reveal impairment in the *later* stages of DAT,[14] such as false positives, intrusion errors, and performance on the BNT, failed to show sufficient impairment in DS compared with controls to enter into the stepwise regression analysis model. When separate comparisons were made for each cognitive variable, several variables, such as intrusion errors, revealed greater impairment in the DS group than in the controls but failed to decline with advancing age among the DS subjects. Intrusion errors occur in a variety of neurologic conditions[28] in addition to AD, and may reflect a more static memory deficit that antedates the onset of more progressive changes of dementia (as seen in older DS adults), such as a decline in the savings score. A decline in short delayed free recall performance with age was not observed among the DS group, perhaps due to a floor effect on this memory function.

There are a number of caveats in the present

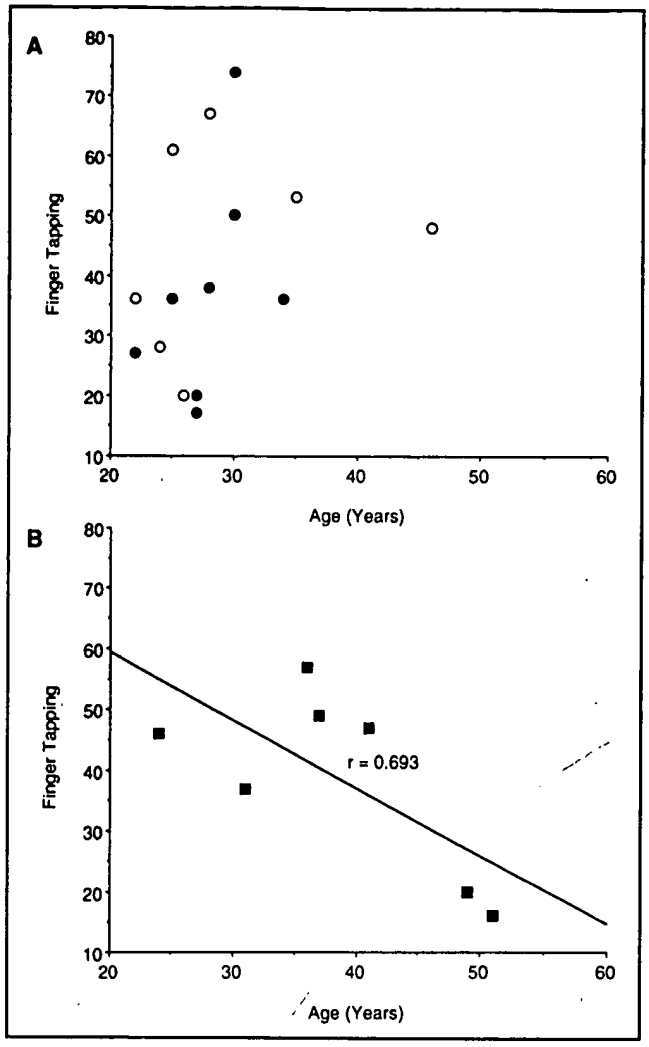

Figure 3. Correlations with age of Finger Tapping (the sum of finger tapping of the dominant and nondominant hands) for each diagnostic group: control (open circles) and memory-nonimpaired Down's (closed circles) subjects in (A) and memory-impaired Down's subjects in (B).

study. First, since DS subjects with severe cognitive impairment could not participate in the neuropsychological testing, the results of the present study apply only to a subpopulation of DS. Second, some of the results of the present study are reported without Bonferroni correction. Third, the study lacked a longitudinal design, which would be necessary to demonstrate a progressive decline of cognitive function among the memory-impaired DS subjects. However, a sensitive marker for DAT[12-15] was employed and revealed an age-dependent decline in performance among DS subjects but not controls, who were within the age range in which the rate of appearance of AD neuropathology in DS is predicted to be greatest.[2]

Impairments in several aspects of verbal function, such as performance on the PPVT, are present in DS subjects compared with controls of similar overall intelligence and age,[16,17] and were also observed in the present study. Impairment of verbal skills appears to precede the development of dementia in DS, since it occurs during childhood years.[16] Consistent with these observations, the DS adults of the present study failed to show an age-dependent decline in performance on the PPVT. Other measures of verbal abilities also failed to discriminate DS from controls or failed to show an age-dependent decline among the DS subjects. Furthermore, the memory-impaired DS group failed to show greater impairment in nonmemory verbal tests than did the controls or the memory-nonimpaired DS group. Therefore, nonmemory verbal tests did not appear to be sensitive in detecting early dementia among DS adults in the present study. Non-DS AD patients generally show impairment on nonmemory verbal tests to a greater extent in advanced rather than early stages of DAT.[14]

If the short delayed savings score is a sensitive marker of early dementia in DS, then DS subjects identified as memory-impaired based on their short delayed savings score performance might also exhibit a decline in performance on other cognitive functions with age. Indeed, a decline in performance on a number of psychometric variables with increasing age that failed to exist for the entire DS group was unmasked among the memory-impaired DS subjects but not among the nonimpaired DS subjects or controls. Similarities in verbal and nonverbal levels of intelligence of these two DS subgroups suggest that any differences between the DS subgroups cannot be attributed simply to a lower baseline level of intelligence among the memory-impaired group. Furthermore, the short delayed savings score, which was used to identify the memory-impaired DS subjects, did not correlate with WAIS-R verbal ($r = 0.238$) or performance ($r = 0.102$) IQ scores but instead correlated with age, which is consistent with the age-dependent development of dementia.

Because the memory-impaired DS group was significantly older than the memory-nonimpaired group, differences between these two DS subgroups might be attributed to age effects rather than to the development of dementia. However, since the short delayed savings score is sensitive in detecting early DAT in AD patients[2-15] and it declined with advancing age among DS adults, then it appears that this savings score is reflecting memory deficits associated with dementia in DS adults who are known to be developing AD neuropathology, rather than reflecting aging. In the present study, the savings score does not decline with advancing age among healthy elderly subjects[29] nor among the non-DS developmentally disabled subjects, in contrast to DS subjects. Furthermore, an age-dependent decline of Logical Memory performance, associated with DAT in the non-DS population, was observed among the memory-impaired nonelderly DS but not among the controls in the present study nor among the nongeriatric (ages 20 to 59) non-DS adults of previous studies.[30] Thus, it appears that differences in cognitive deficits among memory-impaired and nonimpaired DS cannot be sufficiently explained by age effects alone.

An acceleration of neurodegenerative pathology associated with aging might explain the age-dependent decline of performance on nonverbal subtests of the WAIS-R or Finger Tapping on the Halstead-Reitan within the "impaired" subgroup of DS, since these tests are reported to decline with age in the healthy population.[31] Based on numerous reports of the premature onset of a variety of age-dependent phenomena and diseases,[2,32-34] acceleration of aging in DS is believed to exist.

The results of the present study are consistent with the presence of early dementia among DS adults, some of whom are younger than 40 years, and who are within the age range in which the rate of appearance of AD neuropathology is known to be greatest.[2] However, a longitudinal reexamination of the subjects in the present study is required to confirm this hypothesis. Utilization of measures of subtle aspects of memory function, such as the short delayed savings score, should be considered for future studies that attempt to categorize adults with DS into demented and nondemented groups, particularly studies examining potential biochemical correlates of dementia in this population.

Note. Readers can obtain tables 1 and 2 (4 pages) from the National Auxiliary Publications Service, c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY, 10163-3513. Request document no. 05082. Remit with your order (not under separate cover), in US funds only, $7.75 for photocopies or $4.00 for microfiche. Outside the United States and Canada, add postage of $4.50 for the first 20 pages and $1.00 for each 10 pages of material thereafter, or $1.75 for the first microfiche and $.50 for each fiche thereafter. *There is a $15.00 invoicing charge on all orders filled before payment.*

Acknowledgments

Farida Lim for typing sections of the manuscript, Bob Davignon for making the figures, and Kathleen Veinberg, RN, for her technical assistance.

References

1. Heller J. Human chromosome aberrations as related to physical and mental dysfunction. J Hered 1969;60:239-248.
2. Mann D. The pathological association between Down's syndrome and Alzheimer's disease. Mech Ageing Dev 1988;43:99-136.
3. Thase ME, Liss L, Smeltzer D, Maloon J. Clinical evaluation of dementia in Down's syndrome: a preliminary report. J Ment Defic Res 1982;26:239-244.
4. Thase M, Tigner R, Smeltzer D, Liss L. Age-related neuropsychological deficits in Down's syndrome. Biol Psychiatry 1984;19:571-585.
5. Caltagirone C, Nocentini U. Cognitive functions in adult Down's syndrome. Int J Neurosci 1990;54:221-230.
6. Dalton AJ, Crapper DR, Schlotterer GR. Alzheimer's disease in Down's syndrome: visual retention deficits. Cortex 1974;10:366-377.
7. Johanson A, Gustafson L, Brun A, Risberg J, Rosén I, Tideman E. A longitudinal study of dementia of Alzheimer type in Down's syndrome. Dementia 1991;2:159-168.
8. Lai F, Williams RS. A prospective study of Alzheimer's disease in Down syndrome. Arch Neurol 1989;46:849-853.
9. Schapiro MB, Luxenberg JS, Kaye JA, Haxby JV, Friedland RP, Rapoport SI. Serial quantitative CT analysis of brain morphometrics in adult Down's syndrome at different ages. Neurology 1989;39:1349-1353.
10. Percy M, Dalton A, Markovic V, et al. Red cell superoxide dismutase, glutathione peroxidase and catalase in Down syndrome patients with and without manifestations of Alzheimer disease. Am J Med Genet 1990;35:459-467.
11. Delis DC, Massman PJ, Butters N, Salmon D, Cermak LS, Kramer JH. Profiles of demented and amnesiac patients on the California Verbal Learning Test: implications for the assessment of memory disorders. Psychol Assess: J Consult Clin Psychol 1991;3:19-26.
12. Tröster AI, Butters N, Salmon DP, et al. The diagnostic utility of savings score: differentiating Alzheimer's and Huntington's diseases with the Logical Memory and Visual Reproduction tests. J Clin Exp Neuropsychol 1993;15:773-788.
13. Welsh K, Butters N, Hughes J, Mohs R, Heyman A. Detection of abnormal memory decline in mild cases of Alzheimer's disease using CERAD neuropsychological measures. Arch Neurol 1991;48:278-281.
14. Welsh KA, Butters N, Hughes JP, Mohs RC, Heymen A. Detection and staging of dementia in Alzheimer's disease. Arch Neurol 1992;49:448-452.
15. Butters N, Salmon DP, Cullum CM, Cairns P, Tröster AI, Jacobs D. Differentiation of amnesiac and demented patients with the Wechsler Memory Scale-Revised. Clin Neuropsychol 1988;2:133-148.
16. Bellugi U, Bihrle A, Jernigan T, Trauner D, Doherty S. Neuropsychological, neurological and neuroanatomical profile of Williams' syndrome. Am J Med Genet Suppl 1990;6:115-125.
17. Caltagirone C, Nocentini U, Vicari S. Cognitive functions in adult Down's syndrome. Int J Neurosci 1990;54:221-230.
18. Wechsler D. Manual for the Wechsler Adult Intelligence Scale. New York: Psychological Corporation, 1955.
19. Wechsler D. Wechsler memory scale-revised. New York: Psychological Corporation, 1987.
20. Delis DC, Kramer JH, Kaplan E, Ober BA. The California verbal learning test. New York: Psychological Corporation, 1987.
21. Kaplan E, Goodglass H, Weintraub S. Boston naming test. Philadelphia: Lea & Febiger, 1983.
22. Dunn LM, Dunn LM. The Peabody Picture Vocabulary Test-Revised. Manual for forms L & M. Minneapolis, MN: American Guidance Service, 1981.
23. Thorndike RL, Hagen EP, Sattler JM. Stanford-Binet Intelligence Scale: Fourth Edition—technical manual. Chicago: Riverside Publishing, 1986.
24. Benton AL. Differential behavioral effects in frontal lobe disease. Neuropsychologia 1968;6:53-60.
25. Beery KE. Revised administration, scoring and teaching manual for the Developmental Test of Visual-Motor Integration. Cleveland, OH: Modern Curriculum Press, 1982.
26. McCarthy D. Manual for the McCarthy Scales of Children's Abilities. San Antonio, TX: Psychological Corporation, 1972.
27. Reitan RM. The distribution according to age of a psychologic measure dependent upon organic brain functions. J Gerontol 1955;10:338-340.
28. Shindler AG, Caplan LR, Heir DB. Intrusions and preservations. Brain Lang 1984;23:148-158.
29. Haaland KY, Linn RT, Hunt WC, Goodwin JS. A normative study of Russell's variant of the Wechsler Memory Scale in healthy elderly population. J Consult Clin Psychol 1983; 51:878-881.
30. Abikoff H, Alvir J, Hong G, et al. Logical Memory subtest of the Wechsler Memory Scale: age and education norms and alternate-form reliability of two scoring systems. J Clin Exp Neuropsychol 1987;9:435-448.
31. Heaton RK, Grant I, Matthews CG. Differences in neuropsychological test performance associated with age, education, and sex. In: Grant I, Adams K, eds. Neuropsychological assessment of neuropsychiatric disorders. New York: Oxford University Press, 1986:101-120.
32. Brugge KL, Grove GL, Clopton P, Grove MJ, Pracquadio DJ. Evidence for accelerated skin wrinkling among developmentally delayed individuals with Down's syndrome. Mech Ageing Dev 1993;70:213-225.
33. Lott I. Down's syndrome, aging, and Alzheimer's disease: a clinical review. Ann NY Acad Sci 1982;396:15-27.
34. Martin G. Genetics syndrome in man with potential relevance to the pathobiology of aging. In: Bergsma P, Harrison D, eds. Genetic effects on aging. New York: Alan R Liss, 1976:5-39.

American Journal of Pathology, Vol. 142, No. 5, May 1993

Distribution of β/A4 Protein and Amyloid Precursor Protein in Hereditary Cerebral Hemorrhage with Amyloidosis–Dutch Type and Alzheimer's Disease

Annemieke J.M. Rozemuller,*† Raymund A.C. Roos,‡ Gerard T.A.M. Bots,‡ Wouter Kamphorst,* Piet Eikelenboom,† and William E. Van Nostrand§

From the Department of Pathology and the Department of Psychiatry,† Free University Hospital, Amsterdam, The Netherlands; the Departments of Neurology and Neuropathology,‡ University Hospital, Leiden, The Netherlands; and the Department of Microbiology and Molecular Genetics,§ University of California, Irvine, California*

Brain amyloidosis with abundant β/A4 protein deposition in plaques and cortical and meningeal vessels is found in Alzheimer's disease (AD) and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). In contrast to AD, no neuritic pathology or classical congophilic plaques are found in HCHWA-D. Unlike most AD cases, the congophilic angiopathy in HCHWA-D is very severe. It is still unknown whether β/A4 deposits in plaques and vessels have the same origin. In this study, we have used frozen cortical tissue of HCHWA-D and AD patients to investigate the β/A4 amyloid protein and the amyloid precursor protein (APP) in different types of plaques and congophilic angiopathy. Immunohistochemical staining was conducted using antibodies against synthetic β/A4 proteins and antibodies against APP including MAbP2-1, a monoclonal antibody against purified protease nexin-2, which is the secreted form of APP. In contrast to immunohistochemical studies on formalin-fixed, paraffin-embedded tissue, frozen tissue of HCHWA-D patients revealed a very high number of β/A4 plaques resembling AD. All plaques were of the diffuse type. Doublestaining with MAbP2-1 and β/A4 antisera revealed 1) the presence of APP immunoreactivity in classical plaques and transitional forms; 2) the absence of APP immunoreactivity in diffuse plaques in HCHWA-D and AD; and 3) pronounced APP immunoreactivity in congophilic vessels in HCHWA-D in contrast to weak APP staining in congophilic vessels in AD. Together these findings suggest that a) the presence of APP in plaques is related to neuritic changes; b) different processes occur in amyloid formation in plaques and vessels; and c) differences exist between the process of amyloid formation in HCHWA-D and AD. (Am J Pathol 1993, 142: 1449–1457)

Amyloidosis of the central nervous system and formation of paired helical filaments[1] (PHF) are the most characteristic pathological features of patients with Alzheimer's disease (AD)[2] and aged patients with Down Syndrome.[3] The amyloid protein in these disorders is the 4-kd β/A4 protein,[4–7] which is derived from a larger membrane-spanning molecule,[8,9] the amyloid precursor protein (APP).[10–12] The β/A4 protein contains half of the transmembrane region and the first 28 aminoacids of the extracellular domain.[6,7] The normal processing of APP, involved with the release of the extracellular domain known as protease nexin-2 (PN-2),[13–15] is the result of proteolytic cleavage through the β/A4 domain of APP.[16] Releasing of intact β/A4 occurs through aberrant proteolysis of APP caused by unknown brain specific processes.[16,17]

β/A4 deposits are found as senile plaques dispersed in the neuropil of the cortex, hippocampus, and subcortical nuclei. Several types of β/A4 plaques have been described, including the classical amyloid

Supported by the Dutch Praevention fund grant 28-1945 (J.M.R.) and the National Institutes of Health grant AG 00538 (W.E.V.N.).

Accepted for publication October 22, 1992.

Address reprint requests to Dr. Annemieke J.M. Rozemuller, Institute of Pathology, Free University Hospital, Postbox 7057, 1007 MB Amsterdam, The Netherlands.

plaque surrounded by dystrophic neurites and glial cells[18] and the amorphous or diffuse, noncongophilic plaque,[19–28] which seems to be an early stage in amyloid plaque formation.[19,25,26] In addition to amyloid in plaques, amyloid is found in blood vessel walls in some AD patients. Vascular amyloid is localized in the media and adventitia of cortical and meningeal small- and medium-sized arterioles and capillaries.[29–31] This phenomenon is called congophilic angiopathy or cerebral amyloid angiopathy. Plaques and vascular amyloid can also be found in aged nondemented controls, though less abundantly.

Messenger RNA for APP, which is encoded by a gene on chromosome 21,[8,10–12] is expressed in many cell types within and outside the brain.[10,32,33] Recently, mutations in the β/A4 region of the APP protein have been described in a few families with hereditary AD[34–37] but not in other AD families or the sporadic AD cases. In a rare form of brain amyloidosis, called hereditary cerebral hemorrhage with amyloidosis-dutch type (HCHWA-D), severe congophilic angiopathy is found in cortical and meningeal vessels at an early age[38–41] due to a mutation at position 22 of the β/A4 protein.[42–44] In patients with HCHWA-D, a variable number of diffuse, noncongophilic plaques have been described,[38,40] but no neuritic pathology.[40,41,45] Because there is an absence of classical, congophilic plaques in this tissue,[38,40,41] HCHWA-D patients provide a unique model for the study of diffuse plaque formation.

The origin of the β/A4 protein remains unclear. A vascular, glial, neuronal, or extracerebral source has been suggested. It is also unknown whether plaque amyloid and vascular amyloid are induced by the same proteolytic processes. To understand further amyloid formation in plaques and vascular amyloid, we have investigated immunohistochemically the distribution of APP and β/A4 proteins in patients with HCHWA-D and compared the findings with those in AD patients.

Materials and Methods

Brain Tissue

Brain tissue was obtained at autopsy from four HCHWA-D patients, five AD patients, and three controls (see Table 1). AD and control brain tissue was obtained from the Netherlands Brain Bank, Amsterdam (Coordinator Dr. R. Ravid). Diagnosis was confirmed using routine histochemical staining techniques (hematoxylin and eosin staining, Bodian silver, modified Bielschowsky or periodic methenamine silver staining, Congo red staining) performed on formalin-fixed, paraffin-embedded tissue (fixation in 10% formalin for 4 to 5 weeks). Small pieces of parietal, temporal, and/or occipital cortex were frozen in liquid nitrogen for immunohistochemical studies. Immunohistochemical staining was also performed on routine fixed, paraffin-embedded cortical tissue. In addition to numerous plaques, AD patients were selected for the presence of congophilic angiopathy.

Table 1. *Age, Sex, Duration of the Disease, and Clinical Diagnosis of Patients Used in This Study*

Patient No.	Age	Sex	Duration (years)	Diagnosis	Weight (g)
1	81	F	>2	SDAT	990
2	64	M	6	AD	1355
3	40	M	5	AD	1370
4	64	M	7	AD	1210
5	48	M	8	AD	1435
1	47	F	2	HCHWA-D	1250
2	42	M	1 month	HCHWA-D	1500
3	50	M	5	HCHWA-D	1360
4	51	M	3	HCHWA-D	1400
1	73	M	-	Nondemented	1410
2	51	F	-	Nondemented	1190
3	71	F	-	Nondemented	1240

Antibodies

Mouse monoclonal antibodies (MAbs) and antisera in this study included MAbP2-1,[13–15] which recognizes an N-terminal epitope on all major isoforms of APP, and rabbit antiserum APP-45 (gift Dr. K. Beyreuther) against total APP-695, MAb22C11 (Boehringer), rabbit anti-A4-antisera 63122[7] (gift Dr. C.L. Masters and Dr. K. Beyreuther), and SP28[46] (courtesy of Dr. B. Frangione) against respectively 1-42 and 1-28 synthetic β/A4 proteins, NFT200[47] (Innogenetics) against PHF and dystrophic neurites, and a MAb against glial fibrillary acidic protein (Monosan).

Immunohistochemical Staining

Staining with MAbP2-1, APP-45, MAb22C11, anti-A4, SP28, anti-GFAP, and anti-PHF antibodies was performed on frozen tissue. Then 8-μ-thick serial cryostat sections were mounted on poly-L-lysine-coated glass slides, air-dried, and fixed in acetone or a buffer with 4% paraformaldehyde for ten minutes. A4-antisera, APP-45, and MAb22C11 were also used for immunohistochemistry on formalin-fixed tissue. Eight-μ-thick paraffin sections were then mounted on poly-L-lysine- (or chrome-alum

gelatine–) coated glass slides, dehydrated in ethanol, and preincubated in 0.3% H_2O_2 to block endogenous peroxidase. Paraffin sections were pretreated with 85% formic acid[48] before β/A4 staining.

All antibodies were appropriately diluted in phosphate-buffered saline, pH 7.4, containing 1% bovine serum albumin. Immunolabeling for MAbP2-1 was demonstrated with the indirect technique, using peroxidase labeled rabbit anti-mouse antisera (DAKO, Denmark) as a second antibody. Immunolabeling for rabbit antisera (A4, APP-45, SP28) was demonstrated with the peroxidase-antiperoxidase technique.[49] These sections were preincubated with normal swine serum. Peroxidase activity was revealed by the DAB method. (5 mg 3,3-diaminobenzidine [DAB] in 10 ml phosphate-buffered saline, pH 7.4, containing 0.02% H_2O_2 for 3 to 5 minutes). After immunoperoxidase staining, sections were counterstained with Congo red[50] (if not pretreated with formic acid) to visualize the amyloid, then dehydrated, and mounted. Specificity of the polyclonal antiserum APP-45 was confirmed by absorption with purified PN-2/APP. Antiserum APP-45 was incubated overnight at 4 C with several dilutions of PN-2/APP ranging from 1 mmol to 100 mmol and centrifugated at 10,000*g*. The supernatant was used as primary antibody.

Double Staining

Double staining was performed on frozen tissue by incubating simultaneously with anti-β/A4 rabbit polyclonal antibodies and MAbP2-1, followed in the second step by an incubation with alkaline-phosphatase-labeled goat anti-rabbit (Tago, Burlingame, CA) and biotinylated horse anti-mouse (Vector Laboratories, Burlingame, CA). In the third step, sections were incubated with peroxidase-labeled avidin-biotin-complex (Vector Elite kit). Peroxidase activity was visualized using the DAB method. Alkaline phosphatase was revealed using naphthol AS-MX phosphate as substrate and Fast Blue BB as coupling agent. Secondary antisera and reagents were tested for lack of cross-reactivity and nonspecific staining.

Results

Our immunohistochemical findings are summarized in Table 2. In cryostat brain sections of AD, HCHWA-D, and controls, neurons (soma and part of the axon) were immunoreactive with APP-antibodies (Figure 1). Tangle-bearing cells identified by Congo red staining did not show APP-labeling of the intracellular tangles. Glial cells identified by MAbs against glial fibrillary acidic protein, remained unstained for β/A4 or APP. In all cases, a faint staining for APP could be found in cortical and subcortical microvessels.

Plaques

Immunostaining with antibodies against synthetic β/A4, (i.e., anti-A4 [63122] and SP28) showed identical results on adjacent sections. Immunoreactivity was found in numerous plaques in HCHWA-D patients and AD patients. In HCHWA-D patients, the density of β/A4 plaques was much higher in frozen sections than in paraffin sections immunostained for β/A4 or stained using periodic methenamine silver method (40 to 70 plaques/mm^2 and 15 to 40 plaques/mm^2, respectively). Counterstaining with Congo red showed no birefringence of amyloid in HCHWA-D, indicating that these plaques are of the diffuse type (Figure 2). Comparing β/A4 and APP staining in HCHWA-D cases on adjacent sections revealed that diffuse, β/A4-positive plaques did not show reactivity for APP-45 antiserum and MAbP2-1. However, very rare APP-positive deposits were found in two out of four patients. Similarly, brain sections of HCHWA-D patients doublestained with MAbP2-1 and A4 antisera revealed numerous A4-positive but APP-negative plaques (Figure 1). In AD brains, the density of β/A4-immunostained plaques

Table 2. *Immunohistochemical Staining for Antibodies against β/A4 and APP in Diffuse and Classical Plaques, Vascular Amyloid, and Neuropil Threads*

Stain	Diffuse plaques HCHWA-D	AD	Classical plaques HCHWAD-D	AD	Vascular amyloid HCHWA-D	AD	Neuropil threads
Congo red	–	–	np	+	+	+	–
β/A4	+	+	np	+	+	+	–
SP28	+	+	np	+	+	+	–
MAbP2–1	–	–/±	np	+	+	–	–
APP-45	–	–/±	np	+	+	–	–
MAb22C11	–	–/±	np	+	+	–	–

–/±, a few noncongophilic plaques are stained; np = not present.

Figure 1. *Doublestaining for APP (MAbP2-1, brown) and β/A4 (rabbit 63122, blue) on frozen section of a patient with HCHWA-D. Note neuronal staining for APP but absence of APP in diffuse β/A4 plaques. Congophilic vessels are double stained for β/A4 and APP. (Scale bar = 40 μ).*

in frozen tissue was 40 to 80 plaques/mm^2. However, the density of plaques using modified Bielschowsky staining on paraffin sections of adjacent tissue varied between 25 and 70 plaques/mm^2. Counterstaining with Congo red showed that the diffuse, noncongophilic plaques outnumbered the amyloid plaques. Diffuse plaques were found in all cortical layers. Comparison of adjacent sections stained respectively for β/A4 and APP showed that in AD patients 5 to 20% of the β/A4-positive plaques showed APP immunoreactivity either in the corona of classical plaques (Figure 3) or as more diffuse irregular plaque staining. Paraformaldehyde fixation did not reveal more APP-positive plaques than acetone fixation. Doublestaining with β/A4-antisera and MAbP2-1 did not show APP-positive neurites outside the β/A4-positive plaques (Figure 4). Although APP-positive plaques were frequently congophilic, some did not reveal birefringence. Congophilic plaques but not diffuse plaques showed a corona of dystrophic neurites stained with antibodies against PHF. In control cases, some noncongophilic, β/A4-positive plaques were found (0 to 12 plaques/mm^2 in frozen sections). APP-positive deposits were rarely seen. No staining was found in these cases using antibodies against PHF.

Congophilic Angiopathy

Immunohistochemical staining for β/A4 showed reactivity of tunica media and adventitia in many leptomeningeal and cortical microvessels of HCHWA-D patients and less frequently in the microvessels of AD patients. Congo red staining showed less extensive staining of the vessel wall than immunohistochemical demonstration of the β/A4 protein. No

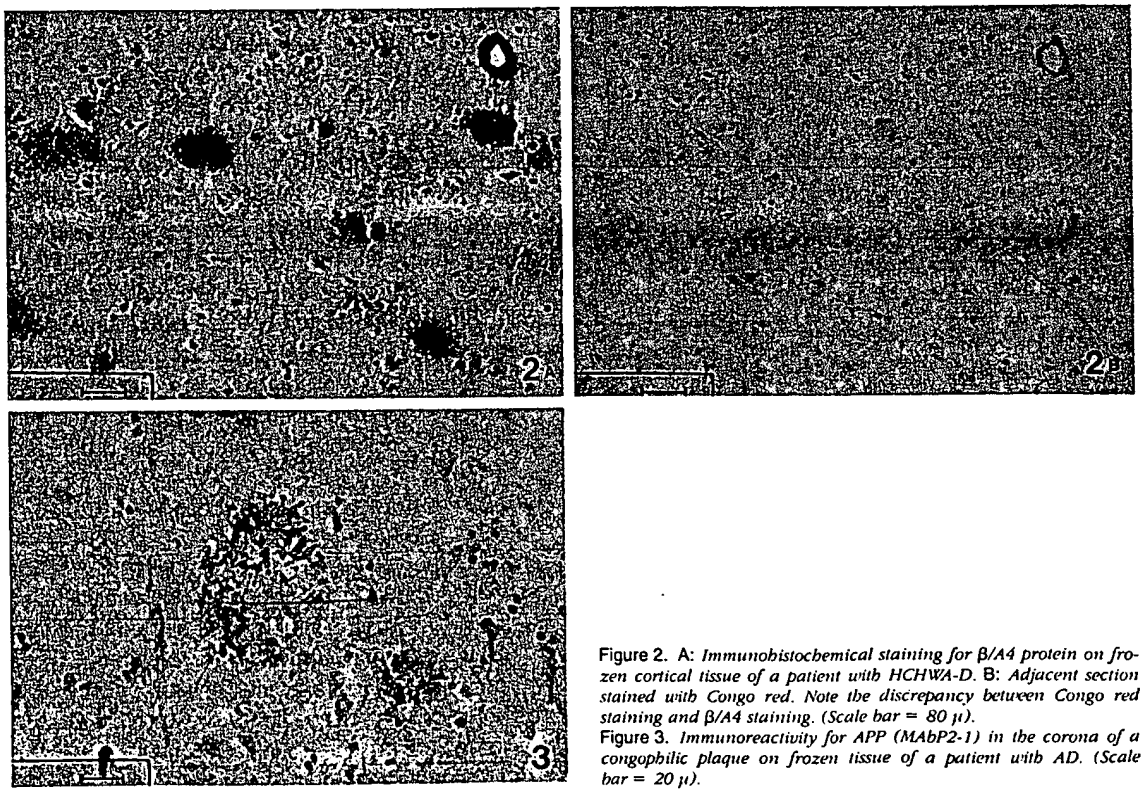

Figure 2. A: *Immunohistochemical staining for β/A4 protein on frozen cortical tissue of a patient with HCHWA-D.* B: *Adjacent section stained with Congo red. Note the discrepancy between Congo red staining and β/A4 staining. (Scale bar = 80 μ).*

Figure 3. *Immunoreactivity for APP (MAbP2-1) in the corona of a congophilic plaque on frozen tissue of a patient with AD. (Scale bar = 20 μ).*

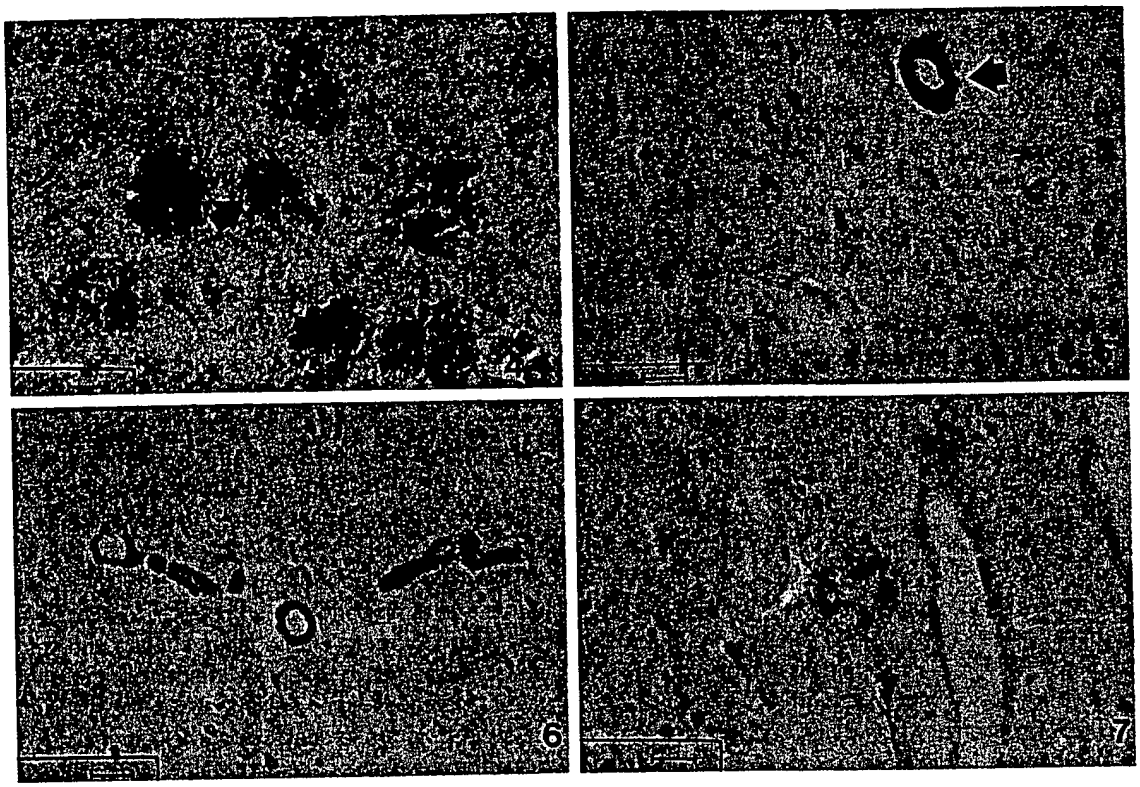

Figure 4. *Doublestaining for APP (MAbP2-1, brown) and β/A4 (rabbit 63122, blue) on frozen tissue of a patient with AD. APP-positive structures are associated with some (arrow) but not all β/A4 plaques. (Scale bar = 40 μ).*
Figure 5. *Staining for APP (MAbP2-1) on frozen tissue of a HCHWA-D patient. Note the difference of immunoreactivity of the amyloidotic vessel (arrow) and the normal vessel. (Scale bar = 20 μ).*
Figure 6. *Immunoreactivity for APP (MAbP2-1) in neurons and in congophilic vessels in a frozen section of a patient with HCHWA-D. (Scale bar = 40 μ).*
Figure 7. *Immunohistochemical staining for APP (MAbP2-1) on a frozen section of an AD patient counterstained with Congo red. Note presence of APP in the corona of a plaque but absence of APP in the adjacent congophilic vessel. (Scale bar = 20 μ).*

relationship was found between the β/A4 plaques and the congophilic vessels. Occasionally, perivascular β/A4 staining was found around congophilic vessels outside the adventitia extending into the neuropil (dyshoric angiopathy). This phenomenon was seen in some AD cases and HCHWA-D cases. In paraffin sections, staining for β/A4 of congophilic vessels was much stronger than plaque staining. This difference, however, was not seen in frozen tissue.

A combination of immunoperoxidase labeling for APP and Congo red staining in frozen sections showed that tunica media and adventitia of most congophilic vessels in HCHWA-D were more intensely stained for the APP protein (Figures 5 and 6). In contrast, amyloidotic vessels in AD patients that were counterstained with Congo red did not show this pronounced APP immunoreactivity (Figure 7). However, in the absence of Congo red counterstaining, weak immunoperoxidase staining for APP could be found in microvessels in AD patients and in controls (data not presented). Furthermore, doublestaining with MAbP2-1 and β/A4 antisera showed doublestained vessels in HCHWA-D (Figure 1) but not in AD. The vessel staining with the polyclonal antiserum was greatly reduced by prior immunoabsorption with PN-2/APP. In addition, staining of parallel sections of HCHWA-D patients with nonimmune rabbit polyclonal antiserum did not show this vessel staining.

Dystrophic Neurites

Staining with NFT200 against PHF visualized numerous tangles and dystrophic neurites (associated with plaques or isolated in the neuropil) in AD but not in HCHWA-D and controls. Dystrophic neurites were especially seen around classical plaques with a congophilic core. These plaques were also associated with APP-positive neurites. In contrast, isolated neuropil threads identified as isolated NFT200-positive threads not associated with plaques were unlabeled with antibodies against

APP both in doublestained sections and compared adjacent sections.

Discussion

The present light-microscopical study shows that APP can not be demonstrated in most diffuse plaques, neither with polyclonal antibodies against total synthetic APP, nor with a MAb against purified PN-2. This was clearly shown in four HCHWA-D patients who lacked congophilic plaques and had only diffuse plaques. Absence of APP in diffuse plaques was also noted in other studies using different antibodies against N- and C-terminal regions of the APP.[45,51–53] Reports about the presence of APP in all plaques in AD[54] including diffuse plaques[55] are probably due to APP immunoreactivity in transitional forms between diffuse and classical plaques. Detection of APP in neurites associated with classical plaques have been demonstrated in several studies.[45,51–54,56] A very small number of APP-positive deposits was found in two out of four HCHWA-D patients, which could suggest that transitional plaque forms are very rare or absent in this disease. On the other hand, a small number of APP deposits without amyloid proteins have also been described in normal controls.[56]

A recent study of AD patients by Tagliavini et al has demonstrated APP immunoreactivity in diffuse plaques with a MAb against the N-terminal region but not with an antiserum against the C-terminal segment.[57] However, the MAbs in our study that are also directed against an N-terminal region of APP[13] did not label the diffuse plaques. Importantly, the study of Tagliavini et al employed formalin fixation and paraffin embedding, whereas we used frozen tissue. It is known that formalin fixation and paraffin embedding can modify the results, especially concerning the presence of serum proteins in plaques.[58] In addition, former studies of HCHWA-D brains performed on paraffin sections[40,45,57] have reported the presence of few plaques in this disorder. In the present study, however, we have found a large number of diffuse plaques that were APP-negative. Comparative β/A4 staining with the same immunohistochemical techniques and antibodies showed that staining on frozen tissue revealed more diffuse plaques/mm^2 in frozen sections than in paraffin sections. Moreover, plaques were stained more intensely in frozen tissue. Similarly, in AD brains, diffuse plaques could be visualised better in frozen tissue. Double labeling for β/A4 and APP in frozen tissue did not show APP-positive cells or neuropil threads outside the β/A4 deposits, with the exception of the neurons. Together, the present findings suggest that previous reports about of APP-positive neurites outside the plaques[59] can be the result of less optimal staining of plaques.

Conflicting reports exist about the presence of APP in congophilic vessels. With the exception of the study of Ko et al,[60] most studies have reported the absence of APP in congophilic vessels in AD using a variety of antibodies.[51–53] A study of Tagliavini et al[45] on cortices of patients with AD and patients with HCHWA-D demonstrated the co-localization of APP and amyloid fibrils in congophilic vessels. However, no discrimination was made between these two diseases. The novel finding in the present study is that congophilic vessels in the HCHWA-D brain exhibit much more APP immune reactivity compared to congophilic vessels in AD. In HCHWA-D, congophilic angiopathy is often severe and starts at an early age, in contrast to AD where congophilic vessels are not always found and vessels are less heavily affected. In addition, quantitative differences may exist between AD and HCHWA-D in the process of amyloid formation in congophilic vessels. The presence of APP in vessels may be a function of the time course of amyloid deposition.

Previous studies have suggested that plaque amyloid derives from the vascular system.[45,61] In the present study, no relationship was found between plaques and congophilic vessels in HCHWA-D nor AD consistent with other reports.[55,62] Several differences between vascular and plaque amyloid have been described.[7,63–65] It has been suggested that diverse processing of the APP molecule occurs in vessel walls and brain parenchyma due to tissue specific enzymes.[63] In a recent ultrastructural study,[66] APP has been found in smooth muscle cells in leptomeningeal vessels, suggesting that smooth muscle cells could be the source of APP in congophilic angiopathy. On the other hand, a neuronal origin of plaque amyloid has been suggested in recent ultrastructural studies.[67,68] Differences between plaque and vascular amyloid are supported by our findings that in HCHWA-D congophilic vessels are immunoreactive for APP, whereas diffuse plaques are unstained.

Reactive neurites, identified by tau antibodies or silver staining, are found in classical plaques and transitional forms but not in diffuse plaques.[19,53] In our opinion, APP-positive structures in some of the plaques are reactive neurites in a later stage of plaque formation. β/A4 deposition is a necessary but not the sole basis for classical plaque forma-

tion.[69,70] In this regard, HCHWA-D can be used as a model for the study of different steps in plaque formation. As there are neither neuritic nor glial changes around the abundant diffuse plaques in HCHWA-D, our findings do not support the hypothesis of Kowall et al[71] that β/A4 itself should produce neurotoxic changes in the neuropil. Perhaps other factors and/or conditions are present in AD, but lacking in HCHWA-D, that are necessary to promote β/A4 neurotoxicity.

In conclusion, we report the absence of APP immunoreactive structures in diffuse plaques both in AD and in HCHWA-D. This suggests that diffuse plaques in HCHWA-D can be used as a model to study very early stages in amyloid plaque formation without neuritic changes. Furthermore, we report a striking difference between HCHWA-D and AD concerning the co-localization of APP in congophilic vessels, suggesting that different processes occur in these two disorders.

Acknowledgments

We thank Drs. C.L. Masters, K. Beyreuther, and B. Frangione for the gift of β/A4 antisera. Brain tissue was obtained from the Netherlands Brain Bank, Amsterdam (Coordinator Dr. R. Ravid).

References

1. Kidd M: Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 1963, 197:192–193
2. Alzheimer A: Ueber eigenartige Krankheitsfälle des späteren Alters. Z f d g Neur u Psych 1911, 4:356–385
3. Jervis GA: Early senile dementia in mongoloid idiocy. Am J Psychiatry 1949, 105:102
4. Glenner GG, Wong CW: Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 1984, 120:885–890
5. Glenner GG, Wong CW: Alzheimer's disease and Down's syndrome: sharing a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun 1984, 122:1131–1135
6. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Beyreuther K: Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. EMBO J 1985, 4:2757–2763
7. Masters CL, Simms G, Weinmann NA, Multhaup G, McDonald BL, Beyreuther K: Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. Proc Natl Acad Sci USA 1985, 82:4245–4249
8. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grezeschik KH, Multhaup G, Beyreuther K, Muller-Hill B: The precursor of Alzheimer disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987, 331:530–532
9. Dyrks T, Weidemann A, Multhaup G, Salbaum JM, Lemaire HG, Kang J, Muller-Hill B, Masters CL, Beyreuther K: Identification, transmembrane orientation and biogenesis of the amyloid A4 precursor of Alzheimer's disease. EMBO J 1988, 7:949–957
10. Tanzi RE, Gusella JF, Watkins PC, Bruns GAP, St. George-Hyslop P, Van Keuren ML, Patterson D, Pajan S, Kurnit DM, Neve RL: Amyloid β-protein gene: cDNA, mRNA distributions and genetic linkage near the Alzheimer locus. Science 1987, 235:880–884
11. Goldgaber D, Lerman MJ, McBride OW, Saffiotti V, Gadjusek DC: Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 1987, 235:877–880
12. Robakis NK, Ramakrishna N, Wolfe G, Wisniewski HM: Molecular cloning and characterization of cDNA encoding the cerebral vascular and the neuritic plaque amyloid peptides. Proc Natl Acad Sci USA 1987, 84: 4190–4194
13. Van Nostrand WE, Wagner SL, Suzuki M, Choi BH, Farrow JS, Geddes JW, Cotman CW, Cunningham DD: Protease nexin-II, a potent antichymotrypsin, shows identity to amyloid β-protein precursor. Nature 1989, 341:546–549
14. Van Nostrand WE, Schmaier AH, Farrow JS, Cunningham DD: Protease Nexin-II (amyloid β-protein precursor): a platelet α-granule protein. Science 1990, 248: 745–748
15. Van Nostrand WE, Wagner SL, Shankle WR, Farrow JS, Dick M, Rozemuller JM, Kuiper MA, Wolters EC, Zimmerland J, Cotman CW, Cunningham DD: Decreased levels of soluble amyloid β-protein precursor in cerebrospinal fluid of live Alzheimer disease patients. Proc Natl Acad Sci USA 1992, 89:2551–2555
16. Sisodia SS, Koo EH, Beyreuther K, Unterbeck A, Price DL: Evidence that β-amyloid protein in Alzheimer's disease is not derived by normal processing. Science 1990, 248:492–495
17. Esch FS, Keim PS, Beattie EC, Blancher RW, Culwell AR, Oltersdorf T, McClure D, Ward PJ: Cleavage of amyloid β peptide during constitutive processing of its precursor. Science 1990, 248:1122–1124
18. Wisniewski HM, Terry RD: Reexamination of the pathogenesis of the senile plaque. Progress in Neuropathology, vol II, 2nd ed. Edited by Zimmerman HM, New York, Grune and Stratton, 1973, pp 1–26
19. Rozemuller JM, Eikelenboom P, Stam FC, Beyreuther K, Masters CL: A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J Neuropathol Exp Neurol 1989, 48:647–663
20. Wisniewski HM, Bancher C, Barcikowska M, Wen GY, Currie J: Spectrum of morphological appearance of

amyloid deposits in Alzheimer's disease. Acta Neuropathol 1989, 78:337–347
21. Braunmühl von A: Alterskrankungen des Zentralnervensystems. Senile Involution. Senile Demenz. Alzheimerkrankheit. Handbuch der spezieller pathologischen Anatomie und Histologie. Nervensystem, 2nd ed. Edited by Scholz W, Berlin, Springer, 1957, pp 337–593
22. Uyematsu S: On the pathology of senile psychosis. The differential diagnostic significance of Redlich-Fischer's miliary plaques. J Nerv Ment Dis 1923, 57:1–260
23. Braak H, Braak E, Ohm T, Bohl J: Alzheimer's disease: mismatch between amyloid plaques and neuritic plaques. Neurosci Lett 1989, 103:24–28
24. Ikeda S-I, Yanagisawa N, Allsop D, Glenner GG: Evidence of amyloid beta-protein immunoreactive early plaque lesions in Down's syndrome brains. Lab Invest 1989, 61:133–137
25. Tagliavini F, Giaccone G, Frangione B, Bugiani O: Preamyloid deposits in the cerebral cortex of patients with Alzheimer's disease and nondemented individuals. Neurosci Lett 1988, 93:191–196
26. Ikeda S-I, Yanagisawa N, Allsop D, Glenner GG: Early senile plaques in Alzheimer's disease demonstrated by histochemistry, immunocytochemistry and electron microscopy. Hum Pathol 1990, 21:1221–1226
27. Ogomori K, Kitamoto T, Tateishi J, Sato Y, Suetsugu M, Abe M: Beta-protein amyloid is widely distributed in the central nervous system of patients with Alzheimer's disease. Am J Pathol 1989, 134:234–251
28. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Ihara Y: A variety of cerebral amyloid deposits in the brains of the Alzheimer-type dementia demonstrated by beta-protein immunostaining. Acta Neuropathol 1988, 76: 541–549
29. Scholz W: Studien zur Pathologie der Hirngefässe. II. Die drusige Entartung der Hirnarterien und -capillaren. Z Neur 1938, 162:694–715
30. Divry G: De l'amyloidose vasculaire cerebrale et meningee (meningopathie amyloide) dans la demence senile. J Belg Neurol 1941, 41/42:141–158
31. Morel F, Wildi E: General and cellular pathochemistry of senile and presenile alterations of the brain. Proceedings of the First International Congress of Neuropathology, vol. II. Edited by Gozzano M. Torino, Italy, Rosenberg and Sellier, 1952, pp 347–374
32. Bahmanyar S, Higgins GA, Goldgaber D, Lewis D, Morrison JH, Wilson MC, Shankar SK, Gajdusek DC: Localization of amyloid beta protein messenger RNA in brains from patients with Alzheimer's disease. Science 1987, 237:77–80
33. Zimmermann K, Herget T, Salbaum JM, Schubert W, Hilbich C, Cramer M, Masters CL, Multhaup G, Kang J: Localization of the putative precursor of Alzheimer's disease-specific amyloid at nuclear envelopes of adult human muscle. EMBO J 1988, 7:367–372
34. Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J: Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 1991, 349:704–706
35. Naruse S, Igarashi S, Aoki K, Kaneko K, Iihara K, Miyatake T, Kobayashi H, Inuzuka T, Shimizu T, Kojima T, Tsuji S: Mis-sense mutation Val-Ile in exon 17 of amyloid precursor protein gene in Japanese familial Alzheimer's disease. Lancet 1991, 337:978–979
36. Yoshioka K, Miki T, Katsuya T, Ogihara T, Sakaki Y: The 717val-Ile substitution in amyloid precursor protein is associated with familial Alzheimer's disease regardless of ethnic groups. Biochem Biophys Res Commun 1991, 178:1141–1146
37. Murrell J, Farlow M, Ghetti B, Benson MD: A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science 1991, 254: 97–99
38. Luyendijk W, Bots GTAM, Vegter-van der Vlis M, Went LN, Frangione B: Hereditary cerebral hemorrhage caused by cortical amyloid angiopathy. J Neurol Sci 1988, 85:267–280
39. Wattendorff A, Bots GTAM, Went LN, Endtz LJ: Familial cerebral amyloid angiopathy presenting as recurrent cerebral haemorrhage. J Neurol Sci 1982, 55: 121–135
40. van Duinen SG, Castano EM, Prelli F, Bots GTAM, Luyendijk W, Frangione B: Hereditary cerebral hemorrhage with amyloidosis in patients of the Dutch origin is related to Alzheimer disease. Proc Natl Acad Sci USA 1987, 84:5991–5994
41. Maat-Schieman MLC, van Duinen SG, Haan J, Roos RAC: Morphology of cerebral plaque-like lesions in hereditary cerebral hemorrhage with amyloidosis (Dutch). Acta Neuropathol 1992, 84:674–679
42. Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, Bots GTAM, Luyendijk W, Frangione B: Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch-type. Science 1990, 248:1124–1126
43. van Broeckhoven C, Haan J, Bakker E, Hardy JA, Hul WV, Vegter-van der Vlis M, Roos RAC: Amyloid β-protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). Science 1990; 248:1120–1122
44. Bakker E, van Broeckhoven C, Haan J, Voorhoeve E, van Hull W, Levy E, Lieberburg I, Carman MD, van Ommen GJB, Roos RAC: DNA diagnosis for hereditary cerebral hemorrhage with amyloidosis (Dutch type). Am J Hum Genet 1991, 49:518–521
45. Tagliavini F, Ghiso J, Timmers WF, Giaccone G, Bugiani O, Frangione B: Coexistence of Alzheimer's amyloid precursor protein and amyloid protein in cerebral vessel walls. Lab Invest 1990, 62:761–766

46. Castaño EM, Ghiso J, Prelli F, Gorevic PD, Migheli A, Frangione B: *In vitro* formation of amyloid fibrils from two synthetic peptides of different lengths homologous to Alzheimer's disease β-protein. Biochem Biophys Res Commun 1986, 141:782–789
47. Gheuens J, Cras P, Perry G, Boons J, Ceuterick-de Groote C, Lübke U, Mercken M, Tabaton M, Gambetti P, Vandermeeren M, Mulvihill P, Siedlak S, Van Heuverswijn H, Martin JJ: Demonstration of a novel neurofilament associated antigen with the neurofibrillary pathology of Alzheimer and related diseases. Brain Res 1991, 558:43–52
48. Kitamoto T, Ogomori K, Tateishi J, Prusiner SB: Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. Lab Invest 1987, 57:230–236
49. Sternberger LA: Immunocytochemistry, 2nd ed. New York, John Wiley & Sons, 1981, pp 104–169
50. Puchtler H, Sweat F, Levine M: On the binding of Congo red by amyloid. J Histochem Cytochem 1962, 10:355–364
51. Shoji M, Hirai S, Yamaguchi H, Harigaya Y, Kawarabayashi T: Amyloid β-protein precursor accumulates in dystrophic neurites of senile plaques in Alzheimer-type dementia. Brain Res 1990, 512:164–168
52. Arai H, Lee VM-Y, Otvos L, Greenberg B, Lowery DE, Sharma SK, Schmidt ML, Trojanowski JQ: Defined neurofilament, tau, and β-amyloid precursor protein epitopes distinguish Alzheimer from non-Alzheimer senile plaques. Proc Natl Acad Sci USA 1990, 87: 2249–2253
53. Joachim CL, Games D, Morris J, Ward P, Frenkel D, Selkoe D: Antibodies to non-beta regions of the beta-amyloid precursor protein detect a subset of senile plaques. Am J Pathol 1991, 138:373–384
54. Ishii T, Kametani F, Haga S, Sato M: The immunohistochemical demonstration of subsequences of the precursor of the amyloid A4 protein in senile plaques in Alzheimer's disease. Neuropathol Appl Neurobiol 1989, 15:135–147
55. Cras P, Kawai M, Siedlak S, Mulvihill P, Gambetti P, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G: Neuronal and microglial involvement in β-amyloid protein deposition in Alzheimer's disease. Am J Pathol 1990, 137:241–246
56. Tate-Ostroff B, Majocha RE, Marotta CA: Identification of cellular and extracellular sites of amyloid precursor protein extracytoplasmatic domain in normal and Alzheimer disease brains. Proc Natl Acad Sci USA 1989, 86:745–749
57. Tagliavini F, Giaccone G, Verga L, Ghiso J, Frangione B, Bugiani O: Alzheimer patients: preamyloid deposits are immunoreactive with antibodies to extracellular domains of the amyloid precursor protein. Neurosci Lett 1991, 128:117–120
58. Rozemuller JM, Eikelenboom P, Kamphorst W, Stam FC: Lack of evidence for dysfunction of the blood-brain barrier in Alzheimer's disease: an immunohistochemical study. Neurobiol Aging 1988, 9:383–391
59. Cras P, Kawai M, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G: Senile plaque neurites in Alzheimer disease accumulate amyloid precursor protein. Proc Natl Acad Sci USA 1991, 88:7552–7556
60. Ko LW, Sheu KR, Blass JP: Immunohistochemical colocalization of amyloid precursor protein with cerebrovascular amyloid of Alzheimer's disease. Am J Pathol 1991, 139:523–533
61. Selkoe DJ: Molecular pathology of amyloidogenic proteins and the role of vascular amyloidosis in Alzheimer's disease. Neurobiol Aging 1989, 10:387–395
62. Kawai M, Kalaria RN, Harik SI, Perry G: The relationship of amyloid plaques to cerebral capillaries in Alzheimer's disease. Am J Pathol 1990, 137:1435–1446
63. Prelli F, Castano EM, Glenner GG, Frangione B: Differences between vascular and plaque core amyloid in Alzheimer's disease. J Neurochem 1988, 51:648–651
64. Joachim CL, Duffy LK, Morris JH, Selkoe DJ: Protein chemical and immunocytochemical studies of meningovascular β-amyloid protein in Alzheimer's disease and normal aging. Brain Res 1988, 474:100–111
65. Rozemuller JM, Abbink J, Stam FC, Hack CE, Eikelenboom P: Distribution pattern and functional state of α1-antichymotrypsin in plaques and vascular amyloid in Alzheimer's disease. Acta Neuropathol 1991, 82: 200–207
66. Shoji M, Hirai S, Harigaya Y, Kawarabayashi T, Yamaguchi H: The amyloid β-protein precursor is localized in smooth muscle cells of leptomeningeal vessels. Brain Res 1990, 530:113–116
67. Martin LJ, Sisodia SS, Koo EH, Cork LC, Dellovade TL, Weidemann A, Beyreuther K, Masters CL, Price DL: Amyloid precursor protein in aged nonhuman primates. Proc Natl Acad Sci USA 1991, 88:1461–1465
68. Cork LC, Masters CL, Beyreuther K, Price DL: Development of senile plaques. Relationships of neuronal abnormalities and amyloid deposits. Am J Pathol 1990, 137:1383–1392
69. Rozemuller JM, Stam FC, Eikelenboom P: Acute phase proteins are present in amorphous plaques in the cerebral but not in the cerebellar cortex in Alzheimer's disease. Neurosci Lett 1990, 119:75–78
70. Eikelenboom P, Rozemuller JM, Fraser H, Berkenbosch F, Kamphorst W, Stam FC: Neuroimmunologic mechanism in cerebral amyloid deposition in Alzheimer's disease. Frontiers of Alzheimer Research. Edited by Ishii T, Allsop D, Selkoe DJ, Amsterdam, Elsevier, 1991, pp 259–271
71. Kowall NW, Beal MF, Busciglio J, Duffy LK, Yankner BA: An *in vivo* model for the neurodegenerative effects of β amyloid and protection by substance P. Proc Natl Acad Sci USA 1991, 88:7247–7251

American Journal of Pathology, Vol. 141, No. 1, July 1992

Short Communication

Light and Electron Microscopic Localization of β-amyloid Protein in Muscle Biopsies of Patients with Inclusion-body Myositis

Valerie Askanas, W. King Engel, and Renate B. Alvarez

From the University of Southern California Neuromuscular Center, the Department of Neurology, University of Southern California School of Medicine, Los Angeles, California

In 11 of 11 inclusion-body myositis (IBM) patients, including one hereditary case, vacuolated muscle fibers contained large and multiple small inclusions immunoreactive for β-amyloid protein (βAP). All IBM muscle biopsies had characteristic cytoplasmic tubulo-filaments (CTFs) by electron microscopy. None of 14 control muscle biopsies contained the βAP immunoreactive (IR) inclusions characteristic of IBM. On the light microscopy level, βAP-IR inclusions colocalized with ubiquitin immunoreactivity. By immunogold electronmicroscopy, βAP immunoreactivity was localized to a) amorphous, poorly defined structures, b) dense floccular material, c) clusters of loosely packed amyloidlike fibrils 6–8 nm in diameter, and d) poorly defined loose fibrillar structures 6–8 nm in diameter. βAP immunoreactive structures were often in proximity to CTFs, but CTFs themselves never contained βAP-IR. Our study provides the first demonstration of βAP accumulations in abnormal human muscle. This finding suggests that in addition to Alzheimer's disease, Down syndrome, and Dutch-type hereditary cerebrovascular amyloidosis, βAP may play an important role in the pathogenesis of other diseases, including ones outside the central nervous system, for example, IBM. (Am J Pathol 1992, 141:31–36)

Inclusion-body myositis (IBM) is diagnosed by a combination of features.[1–6] Clinically adult-onset, usually sporadic, progressive muscle weakness, thinning of the forearms, male predominance, and often a poor or no response to immunosuppression treatment are present. Light-microscopic (LM) pathologic features include: degrees of inflammation varying from abundant to none; muscle fibers with rimmed vacuoles, which usually contain red-staining material with the modified trichrome reaction[7]; and a few atrophic angular, panesterase-dark muscle fibers, suggestive of a denervation component. By routine histochemistry, IBM can be difficult to distinguish from polymyositis. Electronmicroscopy (EM) reveals that abnormal muscle fibers contain cytoplasmic tubulo-filaments (CTFs), 15–21 nm external and 3–6 nm internal diameter; these are the ultrastructural diagnostic criteria of IBM.[1–6]

Autosomal recessive "hereditary IBM" designates rare patients with progressive muscle weakness, CTFs in vacuoles of abnormal muscle fibers, and atrophic muscle fibers, but no inflammation in the biopsy.[8–10] The pathogenesis of these disorders and the origin of CTFs are unknown.

We demonstrated in both sporadic and hereditary IBM that vacuolated muscle fibers contain strong ubiquitin immunoreactivity, which by immunoelectronmicroscopy was localized to CTFs.[11,12] It has also been shown that the vacuolated muscle fibers contain Congo-red positivity indicating amyloid,[13] but the type of amyloid protein was not identified. Because β-amyloid protein (βAP) is localized in ubiquitinated senile plaques in the Alzheimer's disease (AD) brain,[14–17] we investigated whether in IBM muscle biopsies βAP is a constituent of the amyloid deposits that coexist with ubiquitin in vacuolated muscle fibers.

Supported in part by the Norma Bard Research Fund and the Vernon Link Research Fund.

Accepted for publication May 1, 1992.

Address reprint requests to Dr. Valerie Askanas, USC Neuromuscular Center, 637 S. Lucas Ave., Los Angeles, CA 90017.

Material and Methods

Patients

βAP immunolocalization was performed in diagnostic muscle biopsy sections from 25 patients, ages 5–73 years, with the following diagnoses: sporadic IBM, 10; autosomal-recessive hereditary IBM in an Iranian Jew, 1; polymyositis, 7; Duchenne muscular dystrophy, 1; amyotrophic lateral sclerosis, 4; normal muscle, 2. The median age of IBM patients was 64 years and the median age of the non-IBM controls was 60 years. Diagnosis of all patients was based on clinical, laboratory, muscle-biopsy 18-reaction histochemistry,[18] and ultrastructural studies. All IBM patients had CTFs by electronmicroscopy and ubiquitinated inclusions by immunocytochemistry. All patients with sporadic IBM, except the patient with hereditary IBM, had crystal-violet positive (metachromatic red) amyloid inclusions[19] in vacuolated muscle fibers. Crystal-violet positive amyloid inclusions were also positive with thioflavine S.

Immunocytochemistry

Light microscopic immunocytochemistry was done on 10-μm transverse sections of fresh-frozen muscle biopsies, using peroxidase-antiperoxidase (PAP) and fluorescence stainings, following the same general procedures as described.[11,20] Two well-characterized antibodies were used: 1) mouse monoclonal antibody G-OP-1, directed against sequence 8-17 of β-amyloid synthetic peptide,[21] diluted 1:200; and 2) rabbit polyclonal antibody R1280, directed against sequence 1-40 of the synthetic peptide,[22] diluted 1:2000. Ubiquitin (Ub) was localized with a monoclonal antibody, clone 042691GS (Chemicon, Temecula, CA), diluted 1:20. In our previous study, this antibody proved to be specific and produced the same results as several other well-characterized monoclonal and polyclonal Ub antibodies.[11,12,23] Double immunolocalization of βAP and Ub was performed using fluorescence staining, as we have described.[20,23]

Specificity of βAP immunoreactivity was determined by: a) omitting the primary antibody, b) replacing the primary antibody with non-immune serum, and c) absorbing the primary monoclonal antibody with synthetic βAP peptide sequence 8-17.

For electronmicroscopic (EM) immunocytochemistry, βAP was localized on 10-μm unfixed frozen sections adhered to the bottom of 35-mm Petri dishes, according to our method for ultrastructural localization of Ub immunoreactivity.[11] After 48 hours of incubation in the monoclonal antibody against βAP, sections were incubated 69–72 hours in diluted goat anti-mouse-IgG serum conjugated to 10-nm gold (Amersham, Arlington Heights, IL). Then the sections were fixed in paraformaldehyde-glutaraldehyde, postfixed in osmium, and embedded *in situ* in the Petri dish according to our method for cultured muscle.[24] After being embedded, the section in the dish was viewed under phase-contrast microscopy and compared with an adjacent cross-section that had been stained with crystal violet to visualize metachromatic-red amyloid inclusions. The same muscle fibers that contained amyloid-positive inclusions in vacuoles with the crystal violet stain were identified in the adjacent gold-labeled Epon-embedded section. From the latter, a small (1-mm diameter) area containing the identified fiber (or fibers) was marked with a modified 16-gauge needle attached to the microscope.[25] The Epon disk was removed from the dish, and the marked areas were cored-drilled out as described.[24,25] The drilled-out cores, each containing at least one vacuolated muscle fiber, were mounted in an Epon blank block,[25] trimmed, and thin-sectioned. The thin sections were counterstained with uranyl acetate lead citrate, and examined by EM.

Results

Light Microscopy

Vacuolated muscle fibers of sporadic and hereditary IBM biopsies contained dark βAP-IR inclusions within large and small vacuoles (Figure 1). In these positive patients, βAP-IR inclusions were present in almost 100% of their vacuolated fibers. The βAP-IR inclusions had an amorphous pattern and were located subsarcolemmally or more internally in the fibers. At a given cross-sectional level, in hereditary IBM the inclusions tended to be larger and single, whereas in sporadic IBM they were often small and multiple throughout the fiber (Figure 1). In some abnormal and highly vacuolated muscle fibers, βAP-IR accumulations appeared to be extending outside the boundary of the muscle fiber. In sporadic IBM, rarely were there nonvacuolated muscle fibers containing a thin subsarcolemmal rim of βAP-IR (Figure 1A). In the hereditary IBM biopsy, some muscle fibers contained a strong, wide subsarcolemmal βAP accumulation that extended to the interior of the fiber (Figure 1G). Comparison of the immunolocalization of UB and βAP after double immunostaining showed that Ub-IR was colocalized with βAP-IR in all βAP-positive fibers (Figure 1E–H).

When the primary antibody was omitted, absorbed, or replaced by a nonimmune serum, the immunoreaction did not take place.

One hundred percent of the crystal-violet positive muscle fibers had βAP-IR. However, 100% of the βAP-positive muscle fibers in hereditary IBM and 1.5% of the

Figure
chemist reactio βAP im fibers o Immun ulin (F and ul radic I ×813. βAP an

βAP-p
violet
with p
biopsi
istic o

EM I

Most
tibody
mater
(Figur
either
eral p

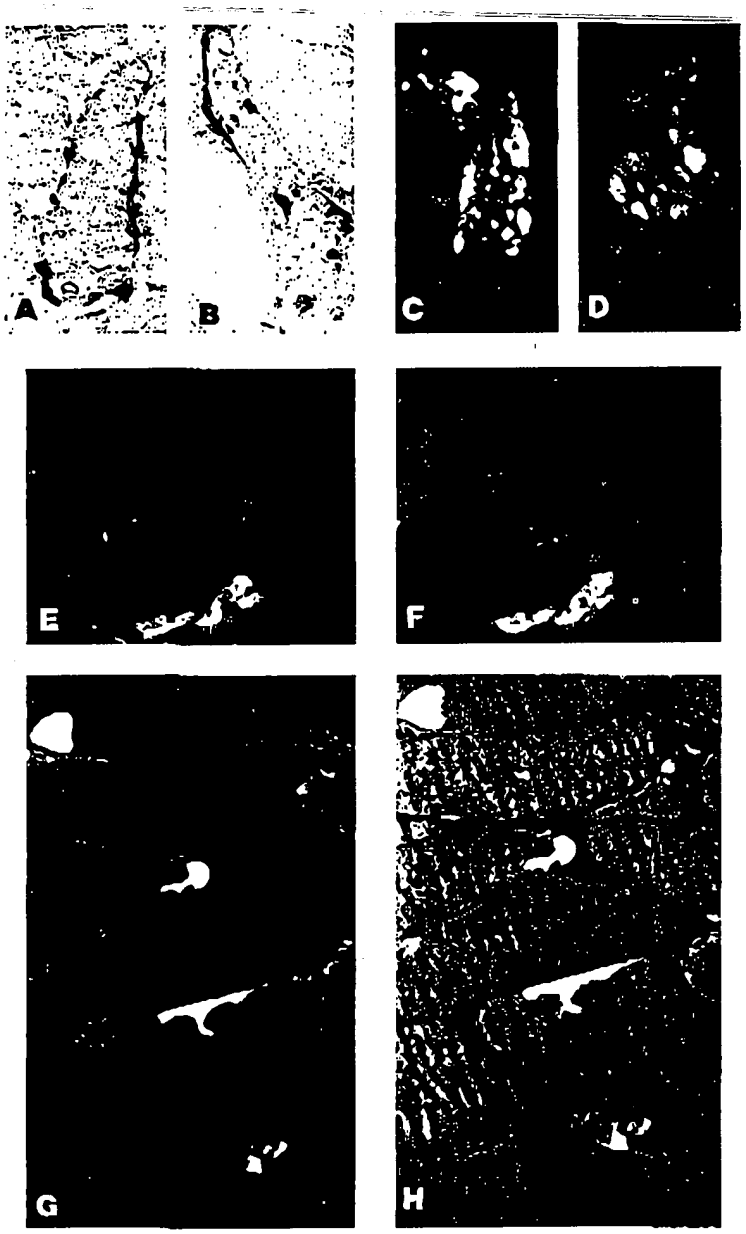

Figure 1. *Light microscopic immunocytochemistry of IBM muscle biopsies.* A,B: *PAP reaction.* C,D: *Fluorescent staining, all of βAP immunoreactivity in vacuolated muscle fibers of sporadic IBM.* ×1250. E–H: *Double immunostaining for βAP* (E,G) *and ubiquitin* (F,H). *βAP is localized with Texas red and ubiquitin with green FITC:* (E,F) *sporadic IBM* ×1250; (G,H) *hereditary IBM* ×813. *There is close colocalization between βAP and ubiquitin in* (E,F) *and* (G,H).

βAP-positive muscle fibers in sporadic IBM were crystal-violet negative. None of the biopsies of seven patients with polymyositis and none of the other control patients' biopsies had the βAP-IR inclusions that were characteristic of IBM.

EM Immunocytochemistry

Most of the structures immunodecorated by the βAP antibody consisted of irregular clusters of nearly amorphous material; those structures were either irregular or rounded (Figure 2A–C,F,I). In such structures, βAP was localized either throughout the entire area or on the looser peripheral parts that aggregated into short thin fibrils 6–8 nm in diameter. βAP-IR was also present on poorly defined dense, floccular material (Figure 2D,E). Amorphous structures intensively immunodecorated by βAP antibody were sometimes seen close to CTFs; however, βAP-IR was never seen on them (Figure 2F). Inside some of the muscle fibers, large clusters of loosely packed amyloid-like fibrils 6–8 nm in diameter had small patches of βAP-IR (Figure 2G). Also immunodecorated by βAP antibody were some loose fibrillar structures (Figure 2H,J). All of these βAP-IR structures seemed to be located inside the muscle fibers. In addition, outside of abnormal muscle fibers, small patches of βAP-IR material were present lying in the extracellular space and intermingled with collagen fibers.

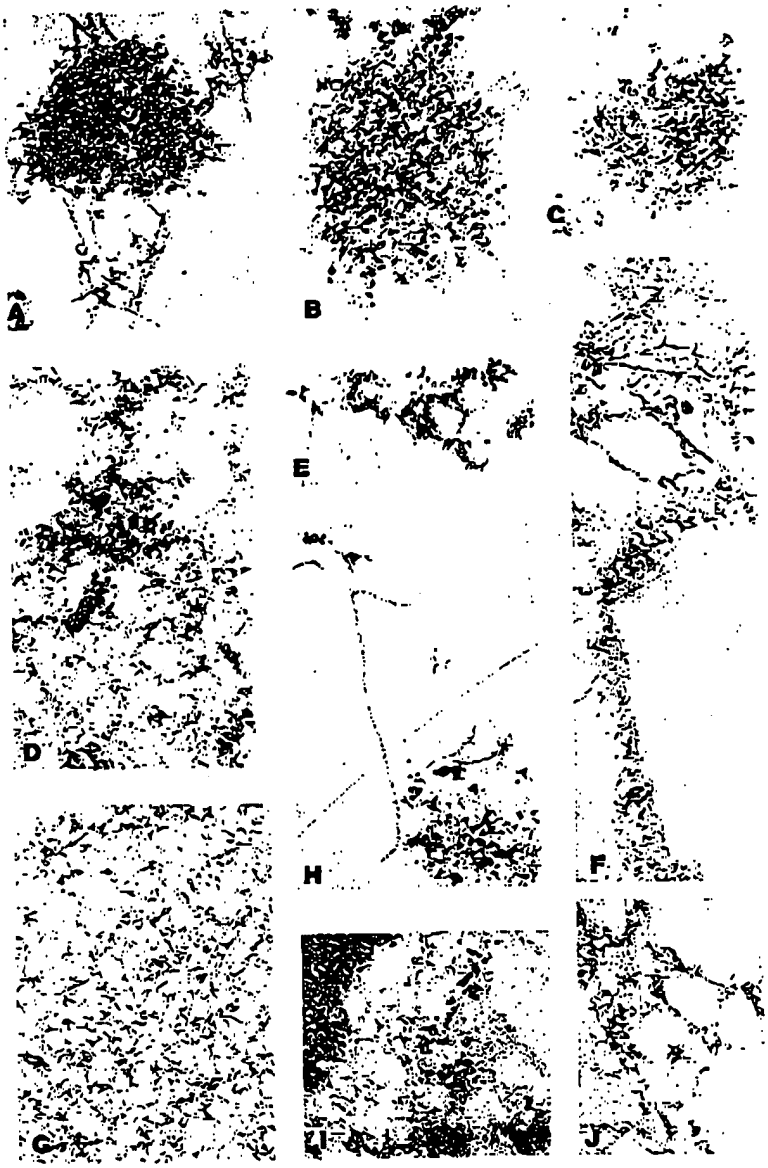

Figure 2. *Electron microscopic immunocytochemistry of βAP using gold particles.* A,B,C,F,I: *Immunodecoration by anti-βAP of various of amorphous or nearly amorphous structures.* A–C: *Gold particles are present on less-tightly packed peripheral material which appears fibrillar.* D,E: *Abundant gold particles are on flocculer material.* G,H,J: *There are small patches of immunodecoration in a cluster of loose fibrillar material. Long, larger diameter (approximately 15 nm) filaments in* (A,E,H) *and a cluster of those filaments in* (F) *are not immunodecorated.* I: *Portion of a myofibril is at the upper left,* (A) ×43,000; (B) ×81,000; (C) ×33,000; (D) ×67,000; (E) ×59,000; (F) ×45,000; (G) ×42,000; (H) ×33,000; (I) ×62,000; (J) ×33,000.

Discussion

This study provides, to our knowledge, the first demonstration of βAP accumulation in diseased human skeletal muscle. It thereby indicates that βAP accumulations are not exclusively in AD, Down syndrome, Dutch-type hereditary cerebrovascular amyloidosis, and advanced age.

βAP was discovered in and first sequenced from the amyloid fibrils in blood vessels of AD patients.[26] Subsequently, it was also isolated from senile plaques of AD brain.[27,28] βAP has received considerable attention regarding the pathogenesis of AD.[16,17,29] βAP is composed of a 4 kda polypeptide, which is produced by proteolytic cleavage of the much larger amyloid precursor protein.[16,17,30]

In AD brain, βAP deposits occur in: a) typical Congo-red positive senile plaques composed mainly of 8-nm diameter amyloid fibrils and dystrophic neurites, and b) so-called diffuse "pre-amyloid" plaques that are Congo-red negative and do not contain typical amyloid fibrils.[31,32] (Congo-red positivity in the form of anisotropic dichroism and crystal-violet positivity in the form of a metachromatic red color are indicators of amyloid, and presumably are based on its β-pleated sheet configuration; they do not indicate the specific type of protein composing the amyloid.) In AD brain, the Congo-red–negative but βAP-positive plaques are considered to be an early pathologic change.[29] Another characteristic of the AD brain, intraneuronal neurofibrillary tangles (NFTs), which are composed of 10-nm paired helical filaments (PHF), lack βAP-IR[15–17,29,33] (although extracellular

"ghost" NTFs do contain βAP-IR.[15,33] Ubiquitin-IR is present not only in both kinds of plaques, Congo-red–positive and negative, but also in the intraneuronal NFTs.[14,15,32,34] Similar abnormalities to those in AD brain occur in brains of older patients with Down syndrome and to a slight extent in advanced aging.[16,17,29]

Because in both IBM muscle and AD brain the amyloid accumulations are immunoreactive with βAP and Ub, they may result from similar cellular events. Electron microscopically, the βAP-IR in IBM is present in structures similar to those described as βAP-positive in AD brain.[15]

Even though by light microscopy βAP-IR and Ub-IR are closely colocalized in IBM muscle fibers, the spatially intermixed abnormal subcellular organelles by electron microscopy show differences: 15–21-nm diameter CTFs are strongly positive for ubiquitin-IR but negative for βAP-IR, whereas the 6–8-nm amyloid-like filaments have prominent βAP-IR (their ubiquitin status has not yet been clarified).

Some muscle fibers had βAP-positive accumulations but, because they were crystal-violet and thioflavin-S negative, apparently did not contain amyloid in β-pleated sheets. Those muscle fibers, widely prevalent in our one case of hereditary IBM, may represent early changes of IBM and therefore be analogous to the finding in AD brains where βAP accumulations in the "diffuse" Congo-red–negative plaques seem to represent early changes.[29]

The presence of βAP accumulations deeply internal in some muscle fibers suggests their intracellular origin. In other vacuolated muscle fibers, the origin of the βAP seems less certain because the fiber appears fragile and the surface membrane might have been transiently broken, such that βAP could have moved in from outside the muscle fiber. By EM, some of the vacuolated fibers contained, seemingly within muscle fibers, a few βAP-IR filaments intermingled with collagen fibrils. Because collagen fibrils are an extracellular component, those βAP filaments may have been generated inside the muscle but become intermixed with exogenous collagen fibrils that had entered through a broken surface membrane, or both may have been generated outside of the fiber.

Future studies will be required to determine the precise origin and pathogenic steps of abnormal βAP accumulation in IBM muscle. Important questions are: a) is βAP in IBM-muscle produced intracellularly or is it transported from the extracellular region, or both; b) is amyloid precursor protein increased in abnormal muscle fibers; c) is the immunoreactive βAP protein of IBM derived from, or does it have homology with, another normal cellular protein, and if so, which one; d) because CTFs (like the intraneuronal NFTs in AD brain[14,15]) contain Ub-immunoreactivity[11] but not βAP-immunoreactivity, does βAP have any relationship to CTF protein; and e) do βAP accumulations occur in any other muscle diseases that we have not yet studied?

Because the accumulations of βAP in IBM muscle and AD brain have many similar features, their pathogenesis may be similar. Accordingly, detailed molecular studies of pathogenic mechanisms in the more readily accessible biopsied living IBM muscle (as compared with brain), including use of cultured IBM muscle biopsies,[35] could be advantageous for understanding both diseases.

Acknowledgments

The authors thank Dr. George G. Glenner for the gift of the βAP monoclonal antibody and synthetic βAP, Dr. Dennis J. Selkoe for the gift of the βAP polyclonal antibody, Burt Handelsman for technical assistance in histochemistry, and Maggie Baburyan for technical assistance in photography.

References

1. Yunis EJ, Samaha FJ: Inclusion body myositis. Lab Invest 1971, 25:240–248
2. Carpenter S, Karpati G, Heller I, Eisen A: Inclusion body myositis: a distinct variety of idiopathic inflammatory myopathy. Neurology 1978, 28:8–17
3. Chou S-M: Myxovirus-like structures in a case of human chronic polymyositis. Science 1967, 158:1453–1455
4. Tomé FMS, Fardeau M, Lebon P, Chevallay M: Inclusion body myositis. Acta Neuropathol 1981, 7:287–291
5. Lotz BP, Engel AG, Nishino H, Stevens JC, Litchy WJ: Inclusion body myositis: observations in 40 patients. Brain 1989, 112:727–747
6. Dalakas MC: Polymyositis, dermatomyositis, and inclusion body myositis. N Engl J Med 1991, 325:1487–1498
7. Engel WK, Cunningham GG: Rapid examination of muscle tissue. An improved trichrome method for fresh-frozen biopsy sections. Neurology 1963, 13:919–923
8. Cole AJ, Kuzniecky R, Karpati G, Carpenter S, Andermann E, Andermann F: Familial myopathy with changes resembling inclusion body myositis and periventricular leukoencephalopathy. Brain 1988, 111:1025–1037
9. Fardeau M, Askanas V, Tomé FMS, Engel WK, Alvarez R, McFerrin J, Chevallay M: Hereditary neuromuscular disorder with inclusion body myositis-like filamentous inclusions: clinical, pathological, and tissue culture studies. Neurology 1990, 40:120
10. Massa R, Weller B, Karpati G, Shonbridge E, Carpenter S: Familial inclusion body myositis among Kurdish-Iranian Jews. Arch Neurol 1991, 48:519–522
11. Askanas V, Serdaroglu P, Engel WK, Alvarez RB: Immunolocalization of ubiquitin in muscle biopsies of patients with inclusion body myositis and oculopharyngeal muscular dystrophy. Neurosci Lett 1991, 130:73–76
12. Askanas V, Serdaroglu P, Engel WK, Alvarez RB: Immuno-

cytochemical localization of ubiquitin in inclusion body myositis allows its light-microscopic distinction from polymyositis. Neurology 1992, 42:460–461

13. Mendell JR, Sahenk Z, Gales T, Paul L: Amyloid filaments in inclusion body myositis. Arch Neurol 1991, 48:1229–1234
14. Perry G, Friedman R, Shaw G, Chan V: Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. Proc Natl Acad Sci 1987, 84:3033–3036
15. Tabaton M, Cammarata S, Mancardi G, Manetto V, Autilio-Gambetti L, Perry G, Gambetti P: Ultrastructural localization of β-amyloid, τ and ubiquitin epitopes in extracellular neurofibrillary tangles. Proc Natl Acad Sci 1991, 88:2089–2102
16. Selkoe DJ: The molecular pathology of Alzheimer's disease. Neuron 1991, 6:487–497
17. Yankner BA, Mesulam MM: β-amyloid and the pathogenesis of Alzheimer's disease. N Engl J Med 1991, 325:1849–1857
18. Engel WK: Muscle biopsies in neuromuscular diseases. Pediatr Clin North Am 1967, 14:963–996
19. Lieb E: Permanent stain for amyloid. Am J Clin Pathol 1947, 17:413–421
20. Askanas V, Bornemann A, Engel WK: Immunocytochemical localization of desmin at human neuromuscular junctions. Neurology 1990, 40:949–953
21. Wong CW, Quaranta V, Glenner GG: Neuritic plaques and cerebrovascular amyloid in Alzheimer's disease are antigenically related. Proc Natl Acad Sci 1985, 82:8729–8732
22. Joachim CL, Mori H, Selkoe DJ: Amyloid β-protein deposition in tissues other than brain in Alzheimer's disease. Nature 1989, 341:226–230
23. Serdaroglu P, Askanas V, Engel WK: Immunocytochemical localization of ubiquitin at human neuromuscular junctions. Neuropathol Appl Neurobiol 1992 (in press)
24. Askanas V, Engel WK: A technique of fiber selection from human muscle tissue cultures for histochemical-electronmicroscopic studies. J Histochem Cytochem 1975, 23:144–146
25. Gorycki M, Askanas V: Improvements of the technique of electron-microscopy of the cultured cells. Stain Technol 1977, 52:249–254
26. Glenner GG, Wong CW: Alzheimer's disease: initial report of the purification and characteristics of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 1984, 120:885–890
27. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyereuther K: Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. Proc Natl Acad Sci 1985, 82:4245–4249
28. Selkoe DJ, Abraham CR, Podlisny MB, Duffy LK: Isolation of low molecular weight proteins from amyloid plaque fibers in Alzheimer's disease. J Neurochem 1986, 146:1820–1834
29. Selkoe DJ: Molecular pathology of amyloidogenic proteins and the role of vascular amyloidosis in Alzheimer's disease. Neurobiol Aging 1989, 10:387–395
30. Kang J, Lemaine HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyereuther K, Muller-Hill B: The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987, 325:733–736
31. Tagliavini F, Giaccone G, Frangione B, Bugiani O: Preamyloid deposits in the cerebral cortex of patients with Alzheimer's disease and nondemented individuals. Neurosci Lett 1988, 93:191–196
32. Yamaguchi H, Hirai S, Morimatsu M, Shoji M, Harigaya Y: Diffuse type of senile plaques in the brains of Alzheimer-type dementia. Acta Neuropathol 1988; 77:113–119
33. Arai H, Lee V M-Y, Otvos Jr. L, Greenberg BD, Lowery DE, Sharma SK, Schmidt ML, Trojanowski JQ: Defined neurofilament, τ, and β-amyloid precursor protein epitopes distinguish Alzheimer from non-Alzheimer senile plaques. Proc Natl Acad Sci USA 1990, 87:2249–2253
34. Suenaga T, Hirano A, Llena JF, Kslezak-Riding H, Yen S-H, Dickson DW: Modified Bielschowsky and immunocytochemical studies on cerebellar plaques in Alzheimer's disease. J Neuropathol Exp Neurol 1990, 49:31–40
35. Alvarez RB, Fardeau M, Askanas V, Engel WK, McFerrin J, Tomé FMS: Characteristic filamentous inclusions reproduced in cultured innervated muscle fibers from patients with familial "inclusion body myositis." J Neurol Sci 1990, 98:178

Sporadic and Familial Cerebral Amyloid Angiopathies

Tamas Revesz[1,2]; Janice L. Holton[1,2]; Tammaryn Lashley[1,2]; Gordon Plant[3]; Agueda Rostagno[4]; Jorge Ghiso[4,5]; Blas Frangione[4,5]

[1] Queen Square Brain Bank, Department of Molecular Pathogenesis and [2]Division of Neuropathology, Institute of Neurology, University College London, London, UK.
[3] National Hospital for Neurology and Neurosurgery, London, UK.
[4] Department of Pathology and [5]Department of Psychiatry, New York University School of Medicine, New York, United States.

Cerebral amyloid angiopathy (CAA) is the term used to describe deposition of amyloid in the walls of arteries, arterioles and, less often, capillaries and veins of the central nervous system. CAAs are an important cause of cerebral hemorrhage and may also result in ischemic lesions and dementia. A number of amyloid proteins are known to cause CAA. The most common sporadic CAA, caused by Aβ deposition, is associated with aging and is a common feature of Alzheimer disease (AD). CAA occurs in several familial conditions, including hereditary cerebral hemorrhage with amyloidosis of Icelandic type caused by deposition of mutant cystatin C, hereditary cerebral hemorrhage with amyloidosis Dutch type and familial AD with deposition of either Aβ variants or wild-type Aβ, the transthyretin-related meningo-vascular amyloidoses, gelsolin as well as familial prion disease-related CAAs and the recently described *BRI2* gene-related CAAs in familial British dementia and familial Danish dementia. This review focuses on the morphological, biochemical, and genetic aspects as well as the clinical significance of CAAs with special emphasis on the *BRI2* gene-related cerebrovascular amyloidoses. We also discuss data relevant to the pathomechanism of the different forms of CAA with an emphasis on the most common Aβ-related types.

Brain Pathol 2002;12:343-357.

Introduction and Historical Perspective

Cerebral amyloid angiopathy (CAA) is a generic morphological term describing the pathological changes occurring in cerebral blood vessels resulting from deposition of amyloid proteins of different origins. Amyloid is the characteristic product of a protein conformational disorder resulting in aggregation, and finally, formation of highly insoluble fibrils. Proteins with the ability for aggregation and polymerization can form amyloid fibrils, which are rich in protein species with β-pleated shett conformation. There are over 20 known proteins or their proteolytic products, which form subunits that assemble into amyloid fibrils, but only a proportion of these proteins deposit in the parenchyma and/or blood vessel walls of the central nervous system (CNS) (32). Amyloid proteins are represented by either wild type or mutated proteins. Mutations may lead to either amino acid substitutions, truncation or elongation of precursor proteins, from which smaller amyloid proteins are cleaved (Table 1).

The first description of CAA in brains of elderly individuals comes from Scholz in 1938 (105), and the presence of CAA was soon confirmed to be a feature of both typical and atypical forms of Alzheimer's disease (for review, see 96). A further interest in CAA arose when it was recognized that this pathology may be responsible for a significant proportion of cerebral hemorrhages occurring in non-hypertensive individuals (122). Further forms of familial CAA-related cerebral hemorrhage such as hereditary cerebral hemorrhage with amyloidosis Icelandic type (HCHWA-I) (12, 67) and hereditary cerebral hemorrhage with amyloidosis Dutch type (HCHWA-D) (65) have also been described. A scientifically significant aspect of CAAs is that the strategy, employing extraction of amyloid fibrils from affected leptomeningeal blood vessels for the identification of the amyloid protein and subsequently the underlying genetic defect, has been a rather fruitful approach. The first amyloid protein to be identified in CAA was cystatin C in HCHWA-I using this approach. This strategy has also resulted in the discovery of the biochemical and genetic abnormalities of a number of other neurological conditions with extensive CAA including HCHWA-D (65) and the *BRI2* gene, or chromosome 13-related, dementias (119, 121). Extracted amyloid fibrils from affected leptomeningeal vessels also led to the discovery of the Aβ peptide (36).

In this paper we review the major morphological and clinical features of CAAs including sporadic, AD-related, and hereditary forms with an emphasis on the recently discovered *BRI2* gene-related cerebrovascular amyloidoses. We also discuss the pathomechanism of

Corresponding author:
Tamas Revesz MD, FRCPath, Division of Neuropathology, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, United Kingdom (e-mail: t.revesz@ion.ucl.ac.uk)

	SPORADIC CAAs	HEREDITARY CAAs							
	SCAA AD	HCHWA-D	FAD	HCHWA-I	FAP/MVA	FAF	PrP-CAA	FBD	FDD
Gene	*AβPP*	*AβPP*	*AβPP, PS1, PS2*	*CYST C*	*TTR*	*GEL*	*PRNP*	*BRI2*	*BRI2*
Chromosome	21	21	21, 14, 1	20	18	9	20	13	13
Precursor molecule	Aβ precursor protein (AβPP)	Aβ precursor protein (AβPP)	Aβ precursor protein (AβPP)	Cystatin C (Cyst C)	Transthyretin (TTR)	Gelsolin (Gel)	Prion protein (PrP)	ABri precursor protein (ABriPP)	ADan precursor protein (ADanPP)
Precursor function	Unknown	Unknown	Unknown	Protease inhibitor	Transport protein	Actin-binding protein	Unknown	Unknown	Unknown
Amyloid protein	Aβ	Aβ	Aβ	ACys	ATTR	AGel	APrP	ABri	ADan

Table 1. *Sporadic and hereditary cerebral amyloid angiopathies (CAAs).*
SCAA = Sporadic cerebral amyloid angiopathy; AD = Alzheimer's disease; HCHWA-D = Hereditary cerebral hemorrhage with amyloidosis-Dutch type; FAD = familial Alzheimer's disease; HCHWA-I = Hereditary cerebral hemorrhage with amyloidosis-Icelandic type; FAP/MVA = Familial amyloid polyneuropathy/meningo-vascular amyloidoses; FAF = Familial amyloidoses Finnish type; PrP-CAA = Prion disease with cerebral amyloid angiopathy; FBD = Familial British dementia; FDD = Familial Danish dementia; *AβPP = Amyloid-β precursor protein* gene; *PS1 = presenilin-1* gene; *PS2 = presenilin-2* gene; *CYST C* = Cystatin C gene; *TTR = Transthyretin* gene; *GEL* = Gelsolin gene; *PRNP* = Prion protein gene; *BRI2 = BRI2* gene; Aβ = Amyloid-β protein; ACys = Amyloid-cystatin C; ATTR = Amyloid-transthyretin; AGel = Amyloid-gelsolin; APrP = Amyloid-Prion protein; ABri = Amyloid-Bri; ADan = Amyloid-Dan.

CAA with particular reference to the most common Aβ-related forms.

Morphological Aspects

In CAA, irrespective of the type of amyloid protein deposited, an acellular thickening of the arterioles, small- and medium-sized arteries, and less often, of capillaries and veins is characteristic. CAA usually affects leptomeningeal arteries and cortical arterioles, but there are exceptions to this general rule (see below). The positive staining of amyloid-laden blood vessels with the Congo red dye with an apple-green color in polarized light and with Thioflavin S or T when the deposits are fluorescent, is considered specific as both methods are dependent on the presence of the β-pleated secondary structure characteristic of amyloid (Figure 1A, E; Figure 3A, B; Figure 5A , B; Figure 6C, F). The evolution of amyloid deposition has been most extensively studied in sporadic CAA. The amyloid primarily deposits in the abluminal portion of the tunica media and adventitia with a tendency to appear first around smooth muscle cells (122). A simple 3-tiered grading system has been recommended for the pathological severity of CAA recognizing "mild," "moderate," and "severe" involvement (127). In "mild" disease, amyloid is restricted to the media of otherwise normal vessels; in "moderate" CAA, the smooth muscle cells are mainly lost and amyloid is arranged in a band with a reticular or radial structure; in "severe" CAA, the vascular architecture is severely disrupted with "double-barreling" and microaneurysm formation. Fibrinoid necrosis of the vessel wall and evidence of perivascular leakage of blood may be seen in "severe" CAA (75, 125).

Ultrastructurally, amyloid fibrils in blood vessels appear as interwoven bundles of 10 nm filaments, which are rather short and arranged in a disorderly way (Figure 4). In vessels showing early changes of CAA, the distribution of the amyloid fibrils may be restricted to the outermost part of the basement membrane at the media-adventitia border in arteries and mostly in the outer portion of the basement membrane around intact smooth muscle cells in smaller sized vessels. Once the deposits have become larger they occupy the abluminal part of the basement membrane and adjacent smooth muscle cells may also show degenerative features (47, 87). The walls of blood vessels severely affected by CAA may be replaced entirely by bundles of such fibrils with loss of smooth muscle cells and a tendency for the amyloid fibrils to radiate from affected capillaries or small arteries into the surrounding neuropil (97,137). However, the

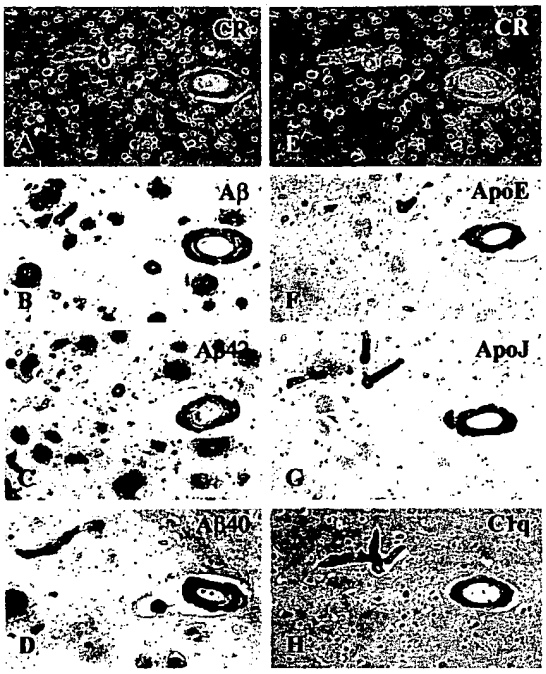

Figure 1. CAA in a case with variant familial Alzheimer's disease with cotton wool plaques caused by mutation of *presenilin-1* (ΔI83/ΔM84). **A.** Congo red preparation showing staining of vessel walls with **(E)** apple-green birefringence in polarized light. Please note the absence of staining in the cotton wool plaques. **B.** An anti-Aβ antibody recognizing amino acids 17-26 stains arteriolar and capillary amyloid together with plaques in cerebral cortex (objective 20). **C.** Aβ42 is deposited in both plaques and blood vessels, while **(D)** Aβ40 predominantly deposits in the latter. **F** and **G.** Amyloid-associated proteins apolipoprotein E and apolipoprotein J are also present primarily in the amyloid-laden blood vessels and deposition of C1q shows a similar pattern **(H)**. (A-H objective ×20)

endothelial cells tend to be preserved even at this advanced stage of the disease process.

Sporadic forms of CAA. CAA is a common neuropathological finding in the brains of elderly individuals with or without evidence of Alzheimer's disease (AD), and not only its incidence but both its extent and severity steadily increases with age (8, 19, 20, 35, 74, 82, 114, 123). In one seminal study, CAA was found in 36% of individuals over 60 years of age and 46% of those over 70 (123). In another study, the frequency of CAA varied between 8% (age 60-69) and 58% (age over 90) (114). The close association between CAA and AD has been shown by several studies demonstrating that CAA is present in over 80% of AD cases, and according to one such study, the CAA is moderate to severe in a quarter

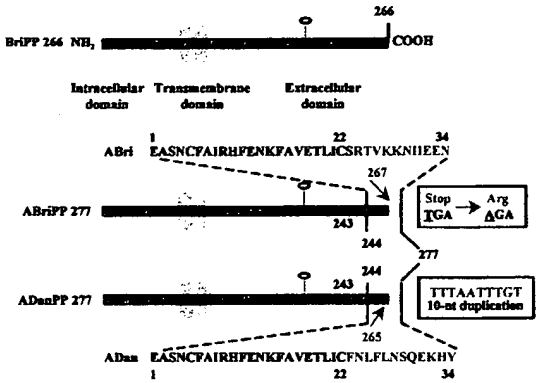

Figure 2. Mutations, amyloid precursor proteins and amyloid peptides in the *BRI2* gene-related diseases. Familial British dementia is characterized by a point mutation while in familial Danish dementia there is a decamer duplication. Both mutations abolish the stop codon resulting in extended precursor proteins from which the amyloid peptides ABri and ADan are cleaved.

of all the AD brains examined (8, 19, 20, 74, 127). In this latter group, a significantly higher frequency of hemorrhages or ischemic lesions was observed compared with those showing little or no amyloid angiopathy (19). In sporadic and AD-related CAA, amyloid deposition usually takes place in leptomeningeal and cortical small arteries and arterioles; when this is significant, the occipital lobe tends to be more severely affected than other cortical areas (114, 123). While CAA may be significant in the cerebellum, areas such as the white matter, basal ganglia, and most of the thalamus are usually spared (85). The most severe clinical consequence of CAA is cerebral hemorrhage, and according to autopsy series, 12 to 15% of all cerebral hemorrhages in the elderly are of this type (52, 63). The high incidence of CAA in AD explains the relatively common (over 5%) occurrence of CAA-related hemorrhage in AD brains (19). In addition to hemorrhage, CAA is known to be associated with a number of other clinical syndromes, including transient neurological symptoms—explained by some as local seizure or spreading depression due to small cortical hemorrhages (41)—and also CAA-associated vasculitis, triggered by vascular Aβ (4, 34). CAA may be a cause of dementia with white matter pathology of the Binswanger's disease type in the background (40, 124). However, co-existent AD is a much more common cause of dementia in sporadic CAA, which is explained by the closely related biologies of the two conditions (41).

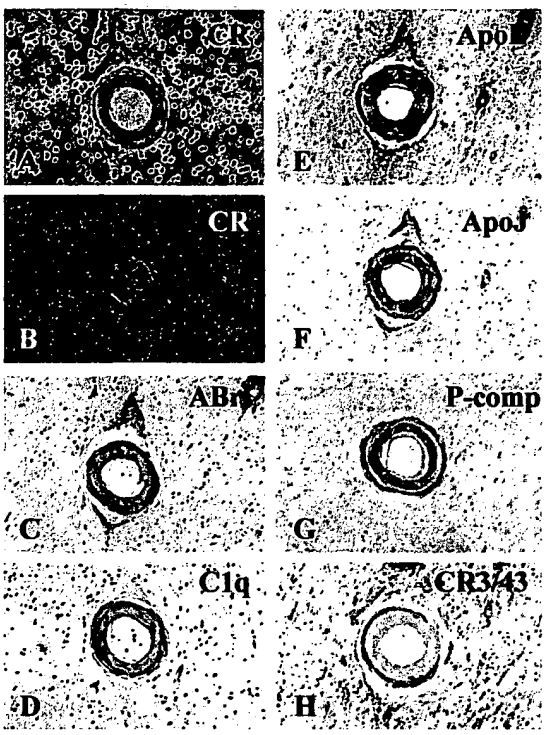

Figure 3. A and B. A small white matter artery affected by severe congophilic angiopathy in familial British dementia. **(C-G)** ABri, C1q, apolipoprotein E, apolipoprotein J and amyloid P component are co-localized. **H.** Activated, MHC Class II-positive microglia in relation to the same amyloid-laden blood vessel **(A-H** objective ×20).

Figure 4. Haphazardly arranged amyloid fibrils, immunogold labeled with an antibody to ABri, expand the basal lamina of a small arteriole (original magnification ×6600).

Aβ, the amyloid subunit in sporadic and AD-related CAAs is a modified, pathogenic form of soluble Aβ (sAβ), which is a constitutive, host protein. Although the 2 proteins have identical sequences, they differ in their secondary structure and physicochemical properties. As opposed to sAβ beta, Aβ is particularly resistant to degradation, and by adopting a β-pleated sheet conformation, readily aggregates and forms fibrils (23). Immunohistochemical and biochemical studies have demonstrated that although the predominant peptide species is $A\beta_{40}$ in sporadic CAA as well as CAAs related to sporadic or familial AD and HCHWA-D (see below), $A\beta_{42}$ is also deposited (11, 39, 53, 78, 101) with higher $A\beta_{42}$ to $A\beta_{40}$ ratios in cortical than in leptomeningeal vessels (101) (Figure 1 B-D). In addition, N-terminally truncated Aβ species with a potential for enhanced aggregation are also present (54, 95, 113). Although the Aβ peptide species are primarily deposited as amyloid to form CAA, monomeric and oligomeric forms of the peptide are also found between and within the cytoplasm of smooth muscle cells in blood vessels (22).

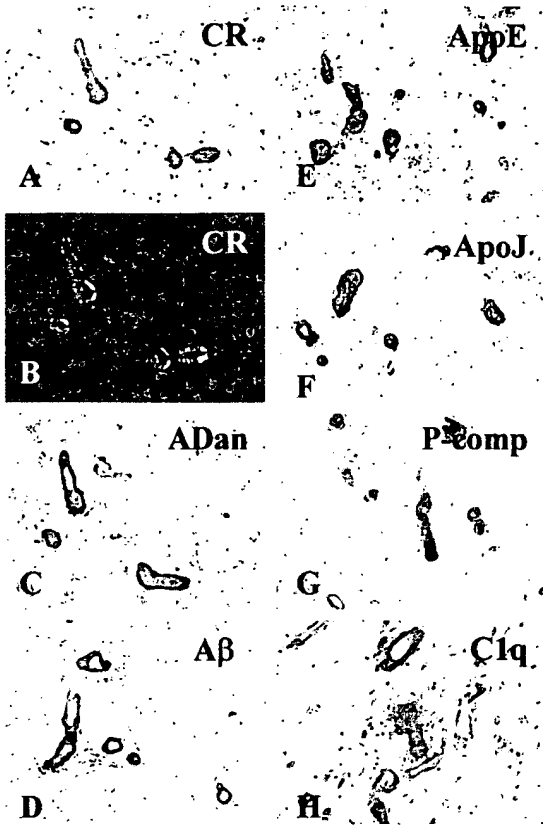

Figure 5. A and B. Amyloid-laden arterioles and capillaries due to **(C)** deposition of ADan. **D.** Many of the blood vessels also contain Aβ. **E-H.** Apolipoprotein E, apolipoprotein J, amyloid P component and C1q are also co-localized **(A-H** objective ×20).

The ϵ4 allele of the *ApoE* gene has emerged as a risk factor not only for sporadic and late onset familial AD (103, 109), but also for sporadic as well as AD-related

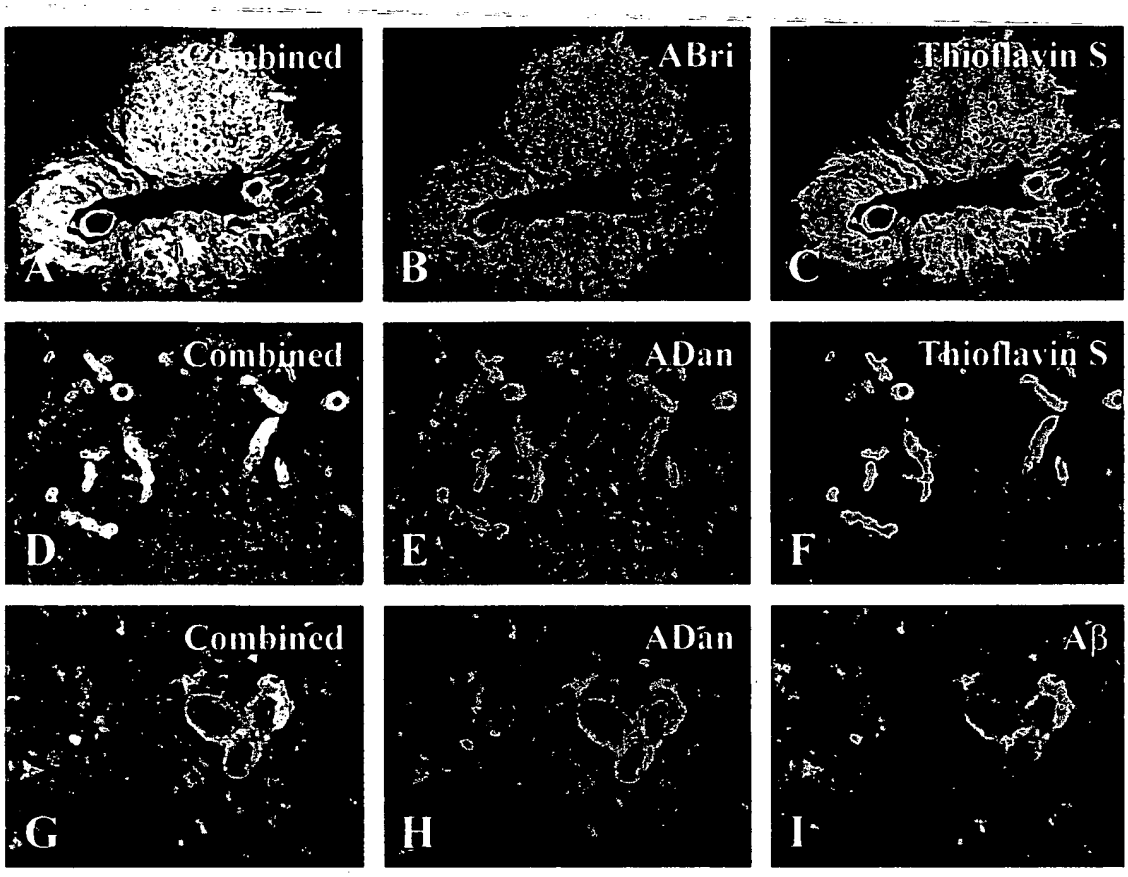

Figure 6. A-C. Confocal images showing co-localization of ABri and Thioflavin S in a small hippocampal vessel with a perivascular plaque, confirming that the amyloid protein is in β-sheet conformation in this lesion (objective ×25). **D-F.** Although ADan co-localizes with Thioflavin S in blood vessels of the subiculum, parenchymal ADan deposits are mostly Thioflavin S negative (preamyloid) (objective ×10). **G-I.** Hippocampal vessels showing a close overlap of ADan and Aβ staining patterns (objective ×25).

CAA (42, 92, 99, 104). In addition, inheritance of the *ApoE* ϵ2 allele has been shown to be associated with CAA-related brain hemorrhage in both AD and non-AD patients (42, 43, 81, 88, 99) The molecular and biochemical mechanisms by which ApoE influences deposition of Aβ in blood vessels and parenchyma remains to be fully understood. There are morphological data to suggest that deposition of ApoE may be the initial step in the disease process followed by deposition of Aβ in blood vessel walls and parenchyma (136). However, the most common finding is the co-localization of ApoE with Aβ in amyloid lesions (109, 133) (Figure 1B, F), and the amount of $A\beta_{40}$ deposited in both brain parenchyma and blood vessels closely correlates with increased copy number of *ApoE* ϵ4 alleles (76, 99). The validity of this latter observation is also underpinned by a study, which—addressing the issue of progression of sporadic CAA from "mild" to "severe" form—demonstrated not only that this process occurs as a result of progressive accumulation of amyloid, primarily $A\beta_{40}$, but is also enhanced by the presence of *ApoE* ϵ4 (3).

There are also now ample experimental in vitro and in vivo data to support the notion that there are critical interactions between ApoE and Aβ. These include those suggesting that ApoE influences the conformation and clearance of Aβ in an isoform and species-dependent manner (5, 49, 137).

Hereditary CAAs. HCHWA-D and familial AD. CAA is a common feature of familial AD caused by mutations of the *amyloid-β precursor protein* (*AβPP*), *presenilin-1*, or *presenilin-2* genes. The mutations of the *AβPP* gene, which is located on chromosome 21, are within or just outside the region encoding for the Aβ pep-

tide (37, 57). As a general rule the clinical phenotype of those mutations found within the coding region of Aβ includes strokes while those outside this region are associated with a more typical AD-type clinical picture (23). The first mutation described in the *AβPP* gene was found within the Aβ region in the autosomal dominant condition HCHWA-D (65). In this condition cerebral hemorrhage is characteristic and is fatal in about two-thirds of the patients with the rest developing multiple strokes resulting in dementia of the vascular type (9). Neuropathologically, severe deposition of amyloid affecting leptomeningeal and cerebral cortical arteries as well as arterioles, and to a lesser extent the cerebellum, is characteristic. Heterogeneous parenchymal Aβ deposits of the diffuse plaque type are also present, but dense plaque cores and neurofibrillary tangles are absent (71). The amyloid subunit in HCHWA-D is homologous to Aβ (see below) containing a single amino acid substitution (glutamine for glutamic acid) at position 22 corresponding to position 693 in the precursor AβPP (65, 116). It has been shown that vascular amyloid deposits in HCHWA-D are composed of both the wild-type Aβ peptide and the Aβ-Q22 variant (98), which forms fibrils at a more accelerated rate than wild-type Aβ and is toxic for cerebral smooth muscle cells as well as endothelial cells (24, 84). Apart from the Dutch mutation, others located within the Aβ sequence, corresponding to codons 692-694 of *AβPP*, are also associated with extensive CAA. The clinical phenotype of the A692G Flemish mutation (46) and of the E693K (Italian) mutation (112) is characterized by dementia as well as cerebral hemorrhage, while dementia is typical for the Arctic, E693G mutation (89). The D694N mutation, described in an Iowa pedigree, is associated with dementia in combination with leukoencephalopathy due to severe CAA (38).

Severe CAA is not limited to pedigrees with particular *AβPP* mutations. In the Volga-German family with AD due to the N141I mutation in the *presenilin-2* gene (64) severe CAA is a prominent feature, and in one case, cerebral hemorrhage is documented (90). Some of the *presenilin-1* mutations are also associated with marked CAA (18, 128, 132, 138). It has recently been suggested that in cases with *presenilin-1* mutations occurring after codon 200, excessive CAA is a distinguishing feature (77). In cases with Δ9 and ΔI83/ΔM84 (Figure 1) mutations of the *presenilin-1* gene, associated with variant AD with spastic paraparesis and cotton wool plaques, extensive CAA is also present (16, 50, 108).

Cystatin C-related familial CAA, HCHWA-I. HCHWA-I, documented in a number of families living in Western Iceland, is an autosomal dominant disorder characterized by severe amyloid deposition within small arteries and arterioles of leptomeninges, cerebral cortex, basal ganglia, brainstem, and cerebellum (44, 55). Although cerebral involvement is the main clinicopathological feature, asymptomatic amyloid deposits are also present in peripheral tissues such as skin, lymphoid tissues, salivary glands, and testes (6, 69). About half of the affected individuals present clinically with a fatal cerebral hemorrhage between 20 to 39 years of age and cognitive decline with dementia may occur in those who survive the hemorrhagic episodes. The amyloid protein deposited in blood vessel walls is 110-residues-long and derived from a mutated form of cystatin C, which contains an amino acid substitution as a result of a single nucleotide change (A for T) at codon 68 of the *cystatin C* gene located on chromosome 20 (30, 67). Cystatin C, produced by many cells (including cortical neurons), is a low molecular weight protein belonging to the type II family of cysteine protease inhibitors (30). It is normally present in biological fluids and in HCHWA-I patients low levels of cystatin C can be demonstrated in the cerebrospinal fluid (CSF) (1, 69). A role for cystatin C in the pathogenesis of other amyloidoses was suggested by the observation that it colocalizes with Aβ in parenchymal and vascular amyloid deposits in AD (68) and that a polymorphism in the *cystatin C* gene may confer a greater risk of developing AD (15, 21).

CAA and transthyretin (meningo-vascular amyloidoses). Inherited amyloidoses of the *transthyretin* (*TTR*) gene are late onset autosomal dominant conditions characterized by deposition of TTR in the extracellular space of several organs (7, 33). TTR is a carrier protein for thyroid hormone and retinol-binding protein in plasma and CSF (102). Variant proteins due to mutations of the *TTR* gene, located on chromosome 18 are associated with amyloid deposition, which has also been documented with wild-type protein (120, 130). The most common clinical phenotype is peripheral sensorimotor neuropathy, which is often associated with autonomic neuropathy (7). Involvement of peripheral organs is a common feature and amyloid deposition can also take place in the vitreous, leptomeninges, and meningeal vessels in some of the variants (7, 94). In the Hungarian (D18G) and Ohio pedigrees (V30G), the clinical and pathological involvement of the meninges and brain parenchyma is prominent, and in the affected members

of the former family the peripheral nerves, organs, and eye are uninvolved (25, 94, 120).

CAA and gelsolin (Familial amyloidosis - Finnish type). Familial amyloidosis-Finnish type (FAF), or gelsolin-related amyloidosis occurring worldwide, is a rare autosomal dominant condition caused by G654A or G654T point mutations of the *gelsolin* gene (45, 60, 66). The clinical presentation is characterized by ophthalmologic, neurologic, and dermatologic manifestations although other symptoms and signs may also be present due to systemic involvement (60). Pathological studies have shown that deposition of gelsolin amyloid takes place in basement membranes and prominent amyloid angiopathy can affect nearly every organ, including the CNS (60, 62). A study of Finnish cases with G654A mutation demonstrated extensive CAA affecting meningeal, cerebral, and spinal blood vessels (62). Gelsolin, an actin-binding protein, occurs in an 80 kDa cytoplasmic and an 83 kDa plasma form, generated from a single gene located on chromosome 9. The secretory form of gelsolin is the sole source of amyloid in gelsolin-related amyloidosis, which is composed of gelsolin fragments spanning positions 173-243 or 173-225 (28, 79). These protein fragments with amino acid substitutions are highly amyloidogenic (80).

CAA and prion protein (PrP). In prion diseases caused by infectious prions, the crucial event is recruitment and conversion of cellular PrP (PrP^{C}) to the pathogenic isoform (PrP^{Sc}), limited proteolysis of which results in a smaller molecule capable of polymerizing into amyloid (100). Of the human prion diseases, which include Creutzfeldt-Jakob disease (CJD), fatal familial insomnia, kuru, variant CJD, and the Gerstmann-Sträussler-Scheinker syndrome (GSS), deposition of PrP amyloid in blood vessels causing CAA is documented only in one pedigree with GSS. This variant is characterized by a T to G mutation at codon 145 of the *PRNP*, resulting in a newly formed stop codon (Y145STOP), which replaces the normally occurring tyrosine at this site. The Y145STOP mutation results in the production of an N- and C-terminally truncated form of PrP consisting of 70 amino acids (26). This particular form of GSS is neuropathologically characterized by deposition of CNS vascular amyloid, immunoreactive with anti-PrP antibodies, in combination with neurofibrillary degeneration. CAA mainly affects the small- and medium-sized vessels of the cerebral and cerebellar grey matter, while the leptomeningeal vessels are less severely affected. Blood vessels of the brainstem or white matter are rarely affected. The presence of perivascular deposits of PrP is also prominent. The capillary involvement is such that the full thickness of affected vessels is usually replaced with PrP amyloid, which is restricted to the adventitia of affected arterioles with relative sparing of the media (26).

Chromosome 13-related CAAs. Familial British dementia (FBD) and familial Danish dementia (FDD) are novel forms of cerebral amyloidosis with extensive CAA.

FBD is the current term (119) for a rare autosomal dominant condition occurring in 3 British pedigrees, originally described as familial presenile dementia with spastic paralysis (135, 134). It is of historical interest that the first pathological description of FBD in 1940 represents the earliest description of CAA in the English language medical literature (134). FBD is clinically characterized by progressive dementia, spastic tetraparesis, and ataxia (83, 97). Cerebral hemorrhage is relatively rare in comparison with some other familial cerebrovascular amyloidoses (83, 97).

FDD was first described using the term heredopathia ophthalmo-oto-encephalica in members of a single Danish pedigree (111, 110). The disease presents in the third decade of life with cataract and ocular hemorrhages, followed some 10 to 20 years later by severe perceptive hearing loss. After the age of 40, cerebellar ataxia commences followed by psychiatric disturbance and progressive dementia (48, 110, 121).

We have described mutations in the *BRI2* gene, also known as *ITM2B*, located on chromosome 13, associated with FBD and FDD (119, 121). The wild type *BRI2* gene encodes a type II, single-spanning transmembrane protein (BriPP), which is composed of 266 amino acids. In FBD, a T→A mutation of the *BRI2* stop codon results in an extended precursor protein 277 amino acids in length (ABriPP) (119). In contrast, FDD is associated with a 10-nt duplication (TTTAA TTTGT) occurring between codons 265 and 266 of the same gene. This results in a frame-shift abolishing the normal stop codon and also produces an extended precursor protein (ADanPP), which, similar to ABriPP, is 277 amino acids long (121) (Figure 2).

Proteolytic cleavage of the mutated precursor proteins in both FBD and FDD at a potential furin cleavage site produces the 34 amino acids long, 4 kDa fragments known respectively as ABri and ADan (59) (Figure 2). Each peptide has a unique C-terminal sequence composed of 12 amino acids, allowing their recognition by specific antibodies (119, 121).

The characteristic features of FBD are amyloid, ultrastructurally fibrillar deposits (Figure 4) in the walls of blood vessels and in the brain parenchyma, in addi-

tion to severe neurofibrillary degeneration. CAA is widespread affecting blood vessels in the leptomeninges and both gray and white matter in almost all regions of the CNS, and such blood vessels stain positively with an antibody recognizing ABri (Figures 3C, 6B). ABri is deposited in amyloid or preamyloid conformation around many blood vessels with CAA, thus forming perivascular plaques (Figure 6 A-C), a finding which is particularly prominent in the cerebellar cortex where it is associated with severe cerebellar degeneration. In addition to CAA, there are abundant parenchymal amyloid plaques and severe neurofibrillary degeneration occurring primarily in limbic areas and including neurofibrillary tangles (NFTs), neuropil threads (NTs), and abnormal neurites (ANs) situated in relation to amyloid plaques and CAA. Binswanger-type white matter degeneration is marked and is thought to be a consequence of ischemia due to CAA (97).

Many of the histological features in FDD, including the presence of widespread ADan CAA (Figures 5, 6 D-F), are similar to those of FBD, but the differences are also remarkable. Among these we found that, in contrast to FBD, the numerous hippocampal ADan deposits are mostly of pre-amyloid rather than amyloid type. The limbic structures also show severe neurofibrillary pathology, and as in FBD, ANs are restricted to areas with amyloid deposition and are therefore largely associated with CAA. In addition to the *BRI2* gene-related conditions, perivascular clustering of argyrophilic and tau-immunoreactive abnormal neurites can also occur in relation to Aβ (93, 118) and prion protein-related CAAs (26). Another difference between FDD and FBD is that in the former the neocortex is more affected by both pre-amyloid peptide deposition and neurofibrillary pathology than in FBD. Retinal pathology, including severe ADan amyloid angiopathy and parenchymal damage, is more severe in FDD than in FBD (48).

FDD cases, which have been available for examination, show a variable amount of Aβ peptide deposition in blood vessels and in brain parenchyma, which may be isolated or combined with ADan (Figures 5C, D and 6G-I). The mechanism and significance of this co-deposition of ADan and Aβ is uncertain (48).

In both FBD and FDD a glial response to the pathology can be demonstrated with activated microglia (Figure 3H) and astrocytes frequently seen in relation to either vascular and parenchymal amyloid deposits, but this response is much less prominent in areas where peptide deposits are of a pre-amyloid nature (47, 48). As has been described in AD (for review, see 2), the vascular and parenchymal amyloid lesions in both FBD and FDD contain components of the classical complement pathway including C1q, C3d, C4d and C5-b9 suggesting in situ complement activation, in addition to other amyloid-associated components (Figures 3 and 5).

Pathomechanism of CAA

The mechanism of amyloid fibril formation together with the origin and tissue specific deposition of amyloid peptides, including Aβ, in cerebral blood vessels and parenchyma are not well-understood. Data from familial AD indicate that the primary structure and concentration of Aβ may be critical factors influencing amyloid fibril formation and that environmental factors and post-translational modification of amino acids may also be important (139). Amyloid-associated proteins such as ApoE, ApoJ, amyloid P component, heparan sulfate proteoglycans, α1-antichymotrypsin and vitronectin may inhibit or promote amyloid formation (14, 31, 70, 117) (Figures 1, 3, 5). The presence of activated microglia in the vicinity of amyloid-laden lesions together with the deposition of complement factors, including C1q and C5b-9 in AD-related CAA (Figure 1H) (2), as well as FBD and FDD (47, 48) (Figures 3D and 5H), suggests a contribution of inflammatory mediators to the degeneration of vascular cells in CAA (117).

A number of mechanisms have been proposed to explain the origin of Aβ in the clinically most prevalent sporadic and AD-related CAAs. These include derivation of cerebral Aβ from the circulation as sAβ is present in the plasma (73, 140). There are in vivo studies suggesting that there is bidirectional transport of Aβ across the blood brain barrier (BBB). This process is receptor-mediated and several receptors, including RAGE (receptor for advanced glycation end-products), SR (scavenger receptor), megalin and LRP1 (low density lipoprotein receptor 1) have been implicated (72, 107, 140, 141). This BBB-modulated bidirectional flow of Aβ suggests that the peripheral peptide can influence the overall CNS catabolic equilibrium. Aβ in CAA may also derive from the CSF, as it can be detected there in both normal individuals and AD patients (27, 51, 106). The arguments against the hematogenous origin of Aβ include that Aβ initially deposits in the adventitia, which is more consistent with an origin of Aβ within the CNS itself (129). The pathological observation that arteries are affected more frequently by amyloid deposition than veins in the subarachnoid space, and that small arteries show CAA more commonly than larger arteries in the same location is used as an argument against Aβ derived from the CSF as the primary source of CAA (129).

Vascular Aβ may originate from vascular smooth muscle cells and/or pericytes (56, 86). A smooth muscle cell origin of Aβ is seemingly supported by several lines of evidence, one of which is morphological showing a close topographical relationship between Aβ deposition and vascular smooth muscle cells (22, 56, 58, 126, 131). Vascular smooth muscle cells have also been shown to express AβPP mRNA (86), and Aβ is able to induce its own production in cultured degenerating cerebrovascular smooth muscle cells (17). However, the presence of amyloid in capillaries suggests that smooth muscle cells are unlikely to be the only source of Aβ in CAA.

Another hypothesis, supported by recent data from morphological observations of human AD brains, proposes that Aβ deposited in the vessel walls is in part or entirely of neuronal origin and is transported along periarterial interstitial fluid drainage pathways to blood vessels (129). In support of this hypothesis is the finding of typical CAA in transgenic animal models of AD, in which neuronal promoters were employed to drive neuronal expression of mutated human AβPP (10, 13, 115).

With regard to the origin of other peptides deposited as CAA, there is indirect evidence to suspect a contribution from blood-derived amyloid proteins in cystatin C, TTR, and gelsolin-related CAAs (7, 61, 91), as these peptides also deposit in peripheral organs. The origin of the amyloid peptides, depositing in CNS blood vessels and parenchyma in FBD and FDD has yet to be established. One can postulate that ABri and ADan could be produced by cellular elements of the CNS as a high level of BriPP mRNA is found in normal human brain (119). However, systemic deposition of ABri also takes place in FBD and elevated levels of ABri are also present in the circulation (no data about this in FDD as yet) (29). These findings raise the possibility that peripheral production of this amyloid peptide could be one of the sources of the CNS amyloid deposits in FBD and perhaps FDD (29). Further studies are required to elucidate these questions.

Conclusions

CAA has emerged as an important aspect of Aβ pathology in elderly individuals and patients suffering from sporadic or familial AD. CAA can result in a variety of cerebral lesions including cerebral hemorrhage of which it is a major cause in elderly individuals. A significant aspect of cerebral amyloid deposition is its relationship to neurodegeneration, and in this respect, new data may emerge from research into novel forms of cerebrovascular amyloidoses, including the *BRI2* gene-related dementias. These latter conditions mimic important pathological aspects of AD in that in addition to CAA, parenchymal amyloid plaques in combination with neurofibrillary degeneration are also a feature. These observations support the notion that different amyloid peptides can result in neurofibrillary degeneration further supporting the idea that amyloid peptides may be of primary importance in the initiation of the neurodegenerative process.

Acknowledgments

This work was supported by NIH grants AG05891 and AG08721, and grants from the Alzheimer Association, Brain Research Trust and the CRDC of RF & UCMS/UCLH.

References

1. Abrahamson M, Barrett AJ, Salvesen G, Grubb A (1986) Isolation of six cysteine proteinase inhibitors from human urine. Their physicochemical and enzyme kinetic properties and concentrations in biological fluids. *J Biol Chem* 261:11282-11289.
2. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE et al (2000) Inflammation and Alzheimer's disease. *Neurobiol Aging* 21:383-421.
3. Alonzo NC, Hyman BT, Rebeck GW, Greenberg SM (1998) Progression of cerebral amyloid angiopathy: accumulation of amyloid-beta40 in affected vessels. *J Neuropathol Exp Neurol* 57:353-359.
4. Anders KH, Wang ZZ, Kornfeld M, Gray F, Soontornniyomkij V, Reed LA, Hart MN, Menchine M, Secor DL, Vinters HV (1997) Giant cell arteritis in association with cerebral amyloid angiopathy: immunohistochemical and molecular studies. *Hum Pathol* 28:1237-1246.
5. Bales KR, Verina T, Dodel RC, Du Y, Altstiel L, Bender M, Hyslop P, Johnstone EM, Little SP, Cummins DJ, Piccardo P, Ghetti B, Paul SM (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat Genet* 17:263-264.
6. Benedikz E, Blondal H, Gudmundsson G (1990) Skin deposits in hereditary cystatin C amyloidosis. *Virchows Arch A Pathol Anat Histopathol* 417:325-331.
7. Benson MD (1996) Leptomeningeal amyloid and variant transthyretins. *Am J Pathol* 148:351-354.
8. Bergeron C, Ranalli PJ, Miceli PN (1987) Amyloid angiopathy in Alzheimer's disease. *Can J Neurol Sci* 14:564-569.
9. Bornebroek M, Haan J, Maat-Schieman ML, van Duinen SG, Roos RA (1996) Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D): I-A review of clinical, radiologic and genetic aspects. *Brain Pathol* 6:111-114.

10. Calhoun ME, Burgermeister P, Phinney AL, Stalder M, Tolnay M, Wiederhold KH, Abramowski D, Sturchler-Pierrat C, Sommer B, Staufenbiel M, Jucker M (1999) Neuronal overexpression of mutant amyloid precursor protein results in prominent deposition of cerebrovascular amyloid. *Proc Natl Acad Sci U S A* 96:14088-14093.
11. Castano EM, Prelli F, Soto C, Beavis R, Matsubara E, Shoji M, Frangione B (1996) The length of amyloid-beta in hereditary cerebral hemorrhage with amyloidosis, Dutch type. Implications for the role of amyloid-beta 1-42 in Alzheimer's disease. *J Biol Chem* 271:32185-32191.
12. Cohen DH, Feiner H, Jensson O, Frangione B (1983) Amyloid fibril in hereditary cerebral hemorrhage with amyloidosis (HCHWA) is related to the gastroentero-pancreatic neuroendocrine protein, gamma trace. *J Exp Med* 158:623-628.
13. Cole GM, Yang F (2000) CAA in transgenic mouse models of Alzheimer's disease. In: *Cerebral Amyloid Angiopathy in Alzheimer's Disease and Related Disorders*, Verbeek MM, de Waal RMW, Vinters HV (eds), Chapter 18, pp. 295-311, Kluwer Academic Publishers, Dordrecht.
14. Coria F, Castano E, Prelli F, Larrondo-Lillo M, van Duinen S, Shelanski ML, Frangione B (1988) Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis. *Lab Invest* 58:454-458.
15. Crawford FC, Freeman MJ, Schinka JA, Abdullah LI, Gold M, Hartman R, Krivian K, Morris MD, Richards D, Duara R, Anand R, Mullan MJ (2000) A polymorphism in the cystatin C gene is a novel risk factor for late-onset Alzheimer's disease. *Neurology* 55:763-768.
16. Crook R, Verkkoniemi A, PerezTur J, Mehta N, Baker M, Houlden H, Farrer M, Hutton M, Lincoln S, Hardy J, Gwinn K, Somer M, Paetau A, Kalimo H, Ylikoski R, Poyhonen M, Kucera S, Haltia M (1998) A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. *Nat Med* 4:452-455.
17. Davis-Salinas J, Saporito-Irwin SM, Cotman CW, Van Nostrand WE (1995) Amyloid beta-protein induces its own production in cultured degenerating cerebrovascular smooth muscle cells. *J Neurochem* 65:931-934.
18. Dermaut B, Kumar-Singh S, De Jonghe C, Cruts M, Lofgren A, Lubke U, Cras P, Dom R, De Deyn PP, Martin JJ, Van Broeckhoven C (2001) Cerebral amyloid angiopathy is a pathogenic lesion in Alzheimer's disease due to a novel presenilin 1 mutation. *Brain* 124:2383-2392.
19. Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D, Heyman A (1996) Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. *Neurology* 46:1592-1596.
20. Esiri MM, Wilcock GK (1986) Cerebral amyloid angiopathy in dementia and old age. *J Neurol Neurosurg Psychiatry* 49:1221-1226.
21. Finckh U, von der KH, Velden J, Michel T, Andresen B, Deng A, Zhang J, Muller-Thomsen T, Zuchowski K, Menzer G, Mann U, Papassotiropoulos A, Heun R, Zurdel J, Holst F, Benussi L, Stoppe G, Reiss J, Miserez AR, Staehelin HB, Rebeck GW, Hyman BT, Binetti G, Hock C, Growdon JH, Nitsch RM (2000) Genetic association of a cystatin C gene polymorphism with late-onset Alzheimer disease. *Arch Neurol* 57:1579-1583.
22. Frackowiak J, Zoltowska A, Wisniewski HM (1994) Non-fibrillar beta-amyloid protein is associated with smooth muscle cells of vessel walls in Alzheimer disease. *J Neuropathol Exp Neurol* 53:637-645.
23. Frangione B, Castano EM, Wisniewski T, Ghiso J, Prelli F, Vidal R (1996) Apolipoprotein E and amyloidogenesis. *Ciba Found Symp* 199:132-141.
24. Frangione B, Revesz T, Vidal R, Holton J, Lashley T, Houlden H, Wood N, Rostagno A, Plant G, Ghiso J (2001) Familial cerebral amyloid angiopathy related to stroke and dementia. *Amyloid* 8 Suppl 1:36-42.
25. Garzuly F, Vidal R, Wisniewski T, Brittig F, Budka H (1996) Familial meningocerebrovascular amyloidosis, Hungarian type, with mutant transthyretin (TTR Asp18Gly). *Neurology* 47:1562-1567.
26. Ghetti B, Piccardo P, Spillantini MG, Ichimiya Y, Porro M, Perini F, Kitamoto T, Tateishi J, Seiler C, Frangione B, Bugiani O, Giaccone G, Prelli F, Goedert M, Dlouhy SR, Tagliavini F (1996) Vascular variant of prion protein cerebral amyloidosis with tau- positive neurofibrillary tangles: the phenotype of the stop codon 145 mutation in PRNP. *Proc Natl Acad Sci U S A* 93:744-748.
27. Ghiso J, Calero M, Matsubara E, Governale S, Chuba J, Beavis R, Wisniewski T, Frangione B (1997) Alzheimer's soluble amyloid beta is a normal component of human urine. *FEBS Lett* 408:105-108.
28. Ghiso J, Haltia M, Prelli F, Novello J, Frangione B (1990) Gelsolin variant (Asn-187) in familial amyloidosis, Finnish type. *Biochem J* 272:827-830.
29. Ghiso J, Holton J, Miravalle L, Calero M, Lashley T, Vidal R, Houlden H, Wood N, Neubert T, Rostagno A, Plant G, Revesz T, Frangione B (2001) Systemic amyloid deposits in familial British dementia. *J Biol Chem* 276:43909-43914.
30. Ghiso J, Jensson O, Frangione B (1986) Amyloid fibrils in hereditary cerebral hemorrhage with amyloidosis of Icelandic type is a variant of gamma-trace basic protein (cystatin C). *Proc Natl Acad Sci U S A* 83:2974-2978.
31. Ghiso J, Plant GT, Revesz T, Wisniewski T, Frangione B (1995) Familial cerebral amyloid angiopathy (British type) with nonneuritic amyloid plaque-formation may be due to a novel amyloid protein. *J Neurol Sci* 129:74-75.
32. Ghiso J, Revesz T, Holton J, Rostagno A, Lashley T, Houlden H, Gibb G, Anderton B, Bek T, Bojsen-Moller M, Wood N, Vidal R, Braendgaard H, Plant G, Frangione B (2001) Chromosome 13 dementia syndromes as models of neurodegeneration. *Amyloid* 8:277-284.
33. Ghiso J, Wisniewski T, Frangione B (1994) Unifying features of systemic and cerebral amyloidosis. *Mol Neurobiol* 8:49-64.

34. Ginsberg L, Geddes J, Valentine A (1988) Amyloid angiopathy and granulomatous angiitis of the central nervous system: a case responding to corticosteroid treatment. *J Neurol* 235:438-440.

35. Glenner GG, Henry JH, Fujihara S (1981) Congophilic angiopathy in the pathogenesis of Alzheimer's degeneration. *Ann Pathol* 1:120-129.

36. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun* 120:885-890.

37. Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC (1987) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* 235:877-880.

38. Grabowski TJ, Cho HS, Vonsattel JP, Rebeck GW, Greenberg SM (2001) Novel amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. *Ann Neurol* 49:697-705.

39. Gravina SA, Ho L, Eckman CB, Long KE, Otvos L, Jr., Younkin LH, Suzuki N, Younkin SG (1995) Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). *J Biol Chem* 270:7013-7016.

40. Gray F, Dubas F, Roullet E, Escourolle R (1985) Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. *Ann Neurol* 18:54-59.

41. Greenberg SM (2000) Clinical aspects and diagnostic criteria of sporadic CAA-related hemorrhage. In: *Cerebral Amyloid Angiopathy in Alzheimer's Disease and Related Disorders*, Verbeek MM, de Waal RMW, Vinters HV (eds), Chapter 1, pp. 3-19, Kluwer Academic Publishers, Dordrecht.

42. Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, Hyman BT (1995) Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. *Ann Neurol* 38:254-259.

43. Greenberg SM, Vonsattel JP, Segal AZ, Chiu RI, Clatworthy AE, Liao A, Hyman BT, Rebeck GW (1998) Association of apolipoprotein E epsilon2 and vasculopathy in cerebral amyloid angiopathy. *Neurology* 50:961-965.

44. Gudmundsson G, Hallgrimsson J, Jonasson TA, Bjarnason O (1972) Hereditary cerebral haemorrhage with amyloidosis. *Brain* 95:387-404.

45. Haltia M, Ghiso J, Prelli F, Gallo G, Kiuru S, Somer H, Palo J, Frangione B (1990) Amyloid in familial amyloidosis, Finnish type, is antigenically and structurally related to gelsolin. *Am J Pathol* 136:1223-1228.

46. Hendriks L, van Duijn CM, Cras P, Cruts M, van Hul W, van Harskamp F, Warren A, McInnis MG, Antonarakis SE, Martin JJ (1992) Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. *Nat Genet* 1:218-221.

47. Holton JL, Ghiso J, Lashley T, Rostagno A, Guerin CJ, Gibb G, Houlden H, Ayling H, Martinian L, Anderton BH, Wood NW, Vidal R, Plant G, Frangione B, Revesz T (2001) Regional distribution of amyloid-Bri deposition and its association with neurofibrillary degeneration in familial British dementia. *Am J Pathol* 158:515-526.

48. Holton JL, Lashley T, Ghiso J, Braendgaard H, Vidal R, Guerin CJ, Gibb G, Hanger DP, Rostagno A, Anderton BH, Strand C, Ayling H, Plant G, Frangione B, Bojsen-Moller M, Revesz T (2002) Familial Danish dementia: a novel form of cerebral amyloidosis associated with deposition of both amyloid-Dan and amyloid-beta. *J Neuropathol Exp Neurol* 61:254-267.

49. Holtzman DM, Bales KR, Wu S, Bhat P, Parsadanian M, Fagan AM, Chang LK, Sun Y, Paul SM (1999) Expression of human apolipoprotein E reduces amyloid-beta deposition in a mouse model of Alzheimer's disease. *J Clin Invest* 103:R15-R21.

50. Houlden H, Baker M, McGowan E, Lewis P, Hutton M, Crook R, Wood NW, Kumar-Singh S, Geddes J, Swash M, Scaravilli F, Holton JL, Lashley T, Tomita T, Hashimoto T, Verkkoniemi A, Kalimo H, Somer M, Paetau A, Martin JJ, Van Broeckhoven C, Golde T, Hardy J, Haltia M, Revesz T (2000) Variant Alzheimer's disease with spastic parapresis and cotton wool plaques is caused by PS-1 mutations that lead to exceptionally high amyloid-beta concentrations. *Ann Neurol* 48:806-808.

51. Ida N, Hartmann T, Pantel J, Schroder J, Zerfass R, Forstl H, Sandbrink R, Masters CL, Beyreuther K (1996) Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J Biol Chem* 271:22908-22914.

52. Itoh Y, Yamada M, Hayakawa M, Otomo E, Miyatake T (1993) Cerebral amyloid angiopathy: a significant cause of cerebellar as well as lobar cerebral hemorrhage in the elderly. *J Neurol Sci* 116:135-141.

53. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y (1994) Visualization of A beta 42(43) and A beta 40 in senile plaques with end- specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron* 13:45-53.

54. Iwatsubo T, Saido TC, Mann DM, Lee VM, Trojanowski JQ (1996) Full-length amyloid-β (1-42(43)) and amino-terminally modified and truncated amyloid-β 42(43) deposit in diffuse plaques. *Am J Pathol*, 149:1823-1830.

55. Jensson O, Palsdottir A, Thorsteinsson L, Arnason A (1989) The saga of cystatin C gene mutation causing amyloid angiopathy and brain hemorrhage--clinical genetics in Iceland. *Clin Genet* 36:368-377.

56. Kalaria RN, Premkumar DR, Pax AB, Cohen DL, Lieberburg I (1996) Production and increased detection of amyloid beta protein and amyloidogenic fragments in brain microvessels, meningeal vessels and choroid plexus in Alzheimer's disease. *Brain Res Mol Brain Res* 35:58-68.

57. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 325:733-736.

58. Kawai M, Kalaria RN, Cras P, Siedlak SL, Velasco ME, Shelton ER, Chan HW, Greenberg BD, Perry G (1993) Degeneration of vascular muscle cells in cerebral amyloid angiopathy of Alzheimer disease. *Brain Res* 623:142-146.

59. Kim SH, Wang R, Gordon DJ, Bass J, Steiner DF, Lynn DG, Thinakaran G, Meredith SC, Sisodia SS (1999) Furin mediates enhanced production of fibrillogenic ABri peptides in familial British dementia. *Nat Neurosci* 2:984-988.

60. Kiuru S (1998) Gelsolin-related familial amyloidosis, Finnish type (FAF), and its variants found worldwide. *Amyloid* 5:55-66.

61. Kiuru S, Matikainen E, Kupari M, Haltia M, Palo J (1994) Autonomic nervous system and cardiac involvement in familial amyloidosis, Finnish type (FAF). *J Neurol Sci* 126:40-48.

62. Kiuru S, Salonen O, Haltia M (1999) Gelsolin-related spinal and cerebral amyloid angiopathy. *Ann Neurol* 45:305-311.

63. Lee SS, Stemmermann GN (1978) Congophilic angiopathy and cerebral hemorrhage. *Arch Pathol Lab Med* 102:317-321.

64. Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu CE, Jondro PD, Schmidt SD, Wang K (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 269:973-977.

65. Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, Bots GT, Luyendijk W, Frangione B (1990) Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* 248:1124-1126.

66. Levy E, Haltia M, Fernandez-Madrid I, Koivunen O, Ghiso J, Prelli F, Frangione B (1990) Mutation in gelsolin gene in Finnish hereditary amyloidosis. *J Exp Med* 172:1865-1867.

67. Levy E, Lopez-Otin C, Ghiso J, Geltner D, Frangione B (1989) Stroke in Icelandic patients with hereditary amyloid angiopathy is related to a mutation in the cystatin C gene, an inhibitor of cysteine proteases. *J Exp Med* 169:1771-1778.

68. Levy E, Sastre M, Kumar A, Gallo G, Piccardo P, Ghetti B, Tagliavini F (2001) Codeposition of cystatin C with amyloid-beta protein in the brain of Alzheimer disease patients. *J Neuropathol Exp Neurol* 60:94-104.

69. Löfberg H, Grubb AO, Nilsson EK, Jensson O, Gudmundsson G, Blöndal H, Arnason A, Thorsteinsson L (1987) Immunohistochemical characterization of the amyloid deposits and quantitation of pertinent cerebrospinal fluid proteins in hereditary cerebral hemorrhage with amyloidosis. *Stroke* 18:431-440.

70. Ma J, Yee A, Brewer HB, Jr., Das S, Potter H (1994) Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* 372:92-94.

71. Maat-Schieman ML, van Duinen SG, Bornebroek M, Haan J, Roos RA (1996) Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D): II-A review of histopathological aspects. *Brain Pathol* 6:115-120.

72. Mackic JB, Stins M, McComb JG, Calero M, Ghiso J, Kim KS, Yan SD, Stern D, Schmidt AM, Frangione B, Zlokovic BV (1998) Human blood-brain barrier receptors for Alzheimer's amyloid-beta 1- 40. Asymmetrical binding, endocytosis, and transcytosis at the apical side of brain microvascular endothelial cell monolayer. *J Clin Invest* 102:734-743.

73. Mackic JB, Weiss MH, Miao W, Kirkman E, Ghiso J, Calero M, Bading J, Frangione B, Zlokovic BV (1998) Cerebrovascular accumulation and increased blood-brain barrier permeability to circulating Alzheimer's amyloid beta peptide in aged squirrel monkey with cerebral amyloid angiopathy. *J Neurochem* 70:210-215.

74. Mandybur TI (1975) The incidence of cerebral amyloid angiopathy in Alzheimer's disease. *Neurology* 25:120-126.

75. Mandybur TI (1986) Cerebral amyloid angiopathy: the vascular pathology and complications. *J Neuropathol Exp Neurol* 45:79-90.

76. Mann DM, Iwatsubo T, Pickering-Brown SM, Owen F, Saido TC, Perry RH (1997) Preferential deposition of amyloidβ protein (Aβ) in the form Aβ40 in Alzheimer's disease is associated with a gene dosage effect of the apolipoprotein E E4 allele. *Neurosci Lett* 221:81-84.

77. Mann DM, Pickering-Brown SM, Takeuchi A, Iwatsubo T (2001) Amyloid angiopathy and variability in amyloid beta deposition is determined by mutation position in presenilin-1-linked Alzheimer's disease. *Am J Pathol* 158:2165-2175.

78. Mann DMA, Iwatsubo T, Ihara Y, Cairns NJ, Lantos PL, Bogdanovic N, Lannfelt L, Winblad B, Maat-Schieman ML, Rossor MN (1996) Predominant deposition of amyloid-beta(42(43)) in plaques in cases of Alzheimer's disease and hereditary cerebral hemorrhage associated with mutations in the amyloid precursor protein gene. *Am J Pathol* 148:1257-1266.

79. Maury CP (1991) Gelsolin-related amyloidosis. Identification of the amyloid protein in Finnish hereditary amyloidosis as a fragment of variant gelsolin. *J Clin Invest* 87:1195-1199.

80. Maury CP, Nurmiaho-Lassila EL, Rossi H (1994) Amyloid fibril formation in gelsolin-derived amyloidosis. Definition of the amyloidogenic region and evidence of accelerated amyloid formation of mutant Asn-187 and Tyr-187 gelsolin peptides. *Lab Invest* 70:558-564.

81. McCarron MO, Nicoll JA (1998) High frequency of apolipoprotein E epsilon 2 allele is specific for patients with cerebral amyloid angiopathy-related haemorrhage. *Neurosci Lett* 247:45-48.

82. McCarron MO, Nicoll JAR (2000) ApoE genotype in relation to sporadic and Alzheimer-related CAA. In: *Cerebral amyloid angiopathy in Alzheimer's disease and related disorders*, Verbeek MM, de Waal RMW, Vinters HV (eds.), Chapter 5, pp. 81-102, Kluwer Academic Publishers, Dordrecht.

83. Mead S, James-Galton M, Revesz T, Doshi RB, Harwood G, Pan EL, Ghiso J, Frangione B, Plant G (2000) Familial British dementia with amyloid angiopathy: early clinical, neuropsychological and imaging findings. *Brain* 123:975-991.

84. Miravalle L, Tokuda T, Chiarle R, Giaccone G, Bugiani O, Tagliavini F, Frangione B, Ghiso J (2000) Substitutions at codon 22 of Alzheimer's abeta peptide induce diverse conformational changes and apoptotic effects in human cerebral endothelial cells. *J Biol Chem* 275:27110-27116.

85. Morris JH (1997) Alzheimer's disease. In: *The neuropathology of dementia*, Esiri MM, Morris JH (eds.), Chapter 4A, pp. 70-121, Cambridge University Press, Cambridge.

86. Natte R, de Boer WI, Maat-Schieman ML, Baelde HJ, Vinters HV, Roos RA, van Duinen SG (1999) Amyloid beta precursor protein-mRNA is expressed throughout cerebral vessel walls. *Brain Res* 828:179-183.

87. Natte R, Yamaguchi H, Maat-Schieman ML, Prins FA, Neeskens P, Roos RA, van Duinen SG (1999) Ultrastructural evidence of early non-fibrillar abeta42 in the capillary basement membrane of patients with hereditary cerebral hemorrhage with amyloidosis, Dutch type. *Acta Neuropathol* 98:577-582.

88. Nicoll JA, Burnett C, Love S, Graham DI, Dewar D, Ironside JW, Stewart J, Vinters HV (1997) High frequency of apolipoprotein E epsilon 2 allele in hemorrhage due to cerebral amyloid angiopathy. *Ann Neurol* 41:716-721.

89. Nilsberth C, Westlind-Danielsson A, Eckman CB, Condron MM, Axelman K, Forsell C, Stenh C, Luthman J, Teplow DB, Younkin SG, Naslund J, Lannfelt L (2001) The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Ab protofibril formation. *Nat Neurosci* 4:887-893.

90. Nochlin D, Bird TD, Nemens EJ, Ball MJ, Sumi SM (1998) Amyloid angiopathy in a Volga German family with Alzheimer's disease and a presenilin-2 mutation (N141I). *Ann Neurol* 43:131-135.

91. Olafsson I, Thorsteinsson L, Jensson O (1996) The molecular pathology of hereditary cystatin C amyloid angiopathy causing brain hemorrhage. *Brain Pathol* 6:121-126.

92. Olichney JM, Hansen LA, Galasko D, Saitoh T, Hofstetter CR, Katzman R, Thal LJ (1996) The apolipoprotein E epsilon 4 allele is associated with increased neuritic plaques and cerebral amyloid angiopathy in Alzheimer's disease and Lewy body variant. *Neurology* 47:190-196.

93. Peers MC, Lenders MB, Defossez A, Delacourte A, Mazzuca M (1988) Cortical angiopathy in Alzheimer's disease: the formation of dystrophic perivascular neurites is related to the exudation of amyloid fibrils from the pathological vessels. *Virchows Arch A Pathol Anat Histopathol* 414:15-20.

94. Petersen RB, Goren H, Cohen M, Richardson SL, Tresser N, Lynn A, Gali M, Estes M, Gambetti P (1997) Transthyretin amyloidosis: a new mutation associated with dementia. *Ann Neurol* 41:307-313.

95. Pike CJ, Overman MJ, Cotman CW (1995) Amino-terminal deletions enhance aggregation of beta-amyloid peptides in vitro. *J Biol Chem* 270:23895-23898.

96. Plant GT, Esiri MM (1997) Familial cerebral amyloid angiopathies. In: *The neuropathology of dementia*, Esiri MM, Morris JH (eds.), Chapter 11, pp. 260-276, Cambridge University Press, Cambridge.

97. Plant GT, Revesz T, Barnard RO, Harding AE, Gautier-Smith PC (1990) Familial cerebral amyloid angiopathy with nonneuritic amyloid plaque formation. *Brain* 113:721-747.

98. Prelli F, Levy E, van Duinen SG, Bots GT, Luyendijk W, Frangione B (1990) Expression of a normal and variant Alzheimer's beta-protein gene in amyloid of hereditary cerebral hemorrhage, Dutch type: DNA and protein diagnostic assays. *Biochem Biophys Res Commun* 170:301-307.

99. Premkumar DR, Cohen DL, Hedera P, Friedland RP, Kalaria RN (1996) Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. *Am J Pathol* 148:2083-2095.

100. Prusiner SB (2001) Shattuck lecture. Neurodegenerative diseases and prions. *N Engl J Med* 344:1516-1526.

101. Roher AE, Lowenson JD, Clarke S, Woods AS, Cotter RJ, Gowing E, Ball MJ (1993) β-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. *Proc Natl Acad Sci U S A* 90:10836-10840.

102. Saraiva MJ (1995) Transthyretin mutations in health and disease. *Hum Mutat* 5:191-196.

103. Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 43:1467-1472.

104. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 90:9649-9653.

105. Scholz W (1938) Studien zur Pathologie der Hirngefässe II. Die drusige Entartung der Hirnarterien und Capillaren. *Z Ges Neurol Psychiat* 162:694-715.

106. Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C (1992) Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature* 359:325-327.

107. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV (2000) Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* 106:1489-1499.

108. Steiner H, Revesz T, Neumann M, Romig H, Grim MG, Pesold B, Kretzschmar HA, Hardy J, Holton JL, Baumeister R, Houlden H, Haass C (2001) A pathogenic presenilin-1 deletion causes abberrant Abeta 42 production in the absence of congophilic amyloid plaques. *J Biol Chem* 276:7233-7239.

109. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* 90:1977-1981.

110. Strömgren E (1981) Heredopathia ophthalmo-oto-encephalica. In: *Handbook of Clinical Neurology*, Vol. 42, Vinken PJ, Bruyn GW (eds.), pp. 150-152, North-Holland Publishing Company, Amsterdam.

111. Strömgren E, Dalby A, Dalby MA, Ranheim B (1970) Cataract, deafness, cerebellar ataxia, psychosis and dementia: A new syndrome. *Acta Neurol Scand* (Suppl 43) 46, 261-262.

112. Tagliavini, F., Rossi, G., Padovani, A., Magoni, M., Andora, G., Sgarzi, M., Bizzi, A., Savoiardo, M., Carella, F., Morbin, M., Giaccone, G., and Bugiani, O (1999) A new βPP mutation related to hereditary cerebral hemorrhage. *Alz Reports* 2, S28.

113. Tekirian TL, Saido TC, Markesbery WR, Russell MJ, Wekstein DR, Patel E, Geddes JW (1998) N-terminal heterogeneity of parenchymal and cerebrovascular A beta deposits. *J Neuropathol Exp Neurol* 57:76-94.

114. Tomonaga M (1981) Cerebral amyloid angiopathy in the elderly. *J Am Geriatr Soc* 29:151-157.

115. Van Dorpe J, Smeijers L, Dewachter I, Nuyens D, Spittaels K, Van Den HC, Mercken M, Moechars D, Laenen I, Kuiperi C, Bruynseels K, Tesseur I, Loos R, Vanderstichele H, Checler F, Sciot R, Van Leuven F (2000) Prominent cerebral amyloid angiopathy in transgenic mice overexpressing the London mutant of human APP in neurons. *Am J Pathol* 157:1283-1298.

116. van Duinen SG, Castano EM, Prelli F, Bots GT, Luyendijk W, Frangione B (1987) Hereditary cerebral hemorrhage with amyloidosis in patients of Dutch origin is related to Alzheimer disease. *Proc Natl Acad Sci U S A* 84:5991-5994.

117. Verbeek MM, Otte-Holler I, Veerhuis R, Ruiter DJ, de Waal RM (1998) Distribution of Aβ-associated proteins in cerebrovascular amyloid of Alzheimer's disease. *Acta Neuropathol* (Berl) 96:628-636.

118. Vidal R, Calero M, Piccardo P, Farlow MR, Unverzagt FW, Mendez E, Jimenez-Huete A, Beavis R, Gallo G, Gomez-Tortosa E, Ghiso J, Hyman BT, Frangione B, Ghetti B (2000) Senile dementia associated with amyloid beta protein angiopathy and tau perivascular pathology but not neuritic plaques in patients homozygous for the APOE-epsilon4 allele. *Acta Neuropathol* (Berl) 100:1-12.

119. Vidal R, Frangione B, Rostagno A, Mead S, Revesz T, Plant G, Ghiso J (1999) A stop-codon mutation in the BRI gene associated with familial British dementia. *Nature* 399:776-781.

120. Vidal R, Garzuly F, Budka H, Lalowski M, Linke RP, Brittig F, Frangione B, Wisniewski T (1996) Meningocerebrovascular amyloidosis associated with a novel transthyretin mis-sense mutation at codon 18 (TTRD 18G). *Am J Pathol* 148:361-366.

121. Vidal R, Revesz T, Rostagno A, Kim E, Holton JL, Bek T, Bojsen-Moller M, Braendgaard H, Plant G, Ghiso J, Frangione B (2000) A decamer duplication in the 3' region of the BRI gene originates an amyloid peptide that is associated with dementia in a Danish kindred. *Proc Natl Acad Sci U S A* 97:4920-4925.

122. Vinters HV (1987) Cerebral amyloid angiopathy. A critical review. *Stroke* 18:311-324.

123. Vinters HV, Gilbert JJ (1983) Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. *Stroke* 14:924-928.

124. Vinters HV, Secor DL, Pardridge WM, Gray F (1990) Immunohistochemical study of cerebral amyloid angiopathy. III. Widespread Alzheimer A4 peptide in cerebral microvessel walls colocalizes with gamma trace in patients with leukoencephalopathy. *Ann Neurol* 28:34-42.

125. Vinters HV, Vonsattel JP (2000) Neuropathologic features and grading of Alzheimer-related and sporadic CAA. In: *Cerebral Amyloid Angiopathy in Alzheimer's Disease and Related Disorders*. Verbeek MM, de Waal RMW, Vinters HV (eds.), Chapter 8, pp.137-155, Kluwer Academic Publishers, Dordrecht.

126. Vinters HV, Wang ZZ, Secor DL (1996) Brain parenchymal and microvascular amyloid in Alzheimer's disease. *Brain Pathol* 6:179-195.

127. Vonsattel JP, Myers RH, Hedley-Whyte ET, Ropper AH, Bird ED, Richardson EP, Jr. (1991) Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study. *Ann Neurol* 30:637-649.

128. Wegiel J, Wisniewski HM, Kuchna I, Tarnawski M, Badmajew E, Popovitch E, Kulczycki J, Dowjat WK, Wisniewski T: Cell-type-specific enhancement of amyloid-beta deposition in a novel presenilin-1 mutation (P117L). *J Neuropathol Exp Neurol* 1998, 57:831-838.

129. Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM, Roher AE (1998) Cerebral amyloid angiopathy - Amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am J Pathol* 153:725-733.

130. Westermark P, Sletten K, Johansson B, Cornwell GG, 3rd (1990) Fibril in senile systemic amyloidosis is derived from normal transthyretin. *Proc Natl Acad Sci U S A* 87:2843-2845.

131. Wisniewski HM, Wegiel J, Kotula L (1996) Review. David Oppenheimer Memorial Lecture 1995: Some neuropathological aspects of Alzheimer's disease and its relevance to other disciplines. *Neuropathol Appl Neurobiol* 22:3-11.

132. Wisniewski T, Dowjat WK, Buxbaum JD, Khorkova O, Efthimiopoulos S, Kulczycki J, Lojkowska W, Wegiel J, Wisniewski HM, Frangione B (1998) A novel Polish presenilin-1 mutation (P117L) is associated with familial Alzheimer's disease and leads to death as early as the age of 28 years. *Neuroreport* 9:217-221.

133. Wisniewski T, Frangione B (1992) Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci Lett* 135:235-238.

134. Worster-Drought C, Greenfield JG, McMenemey WH (1940) A form of familial presenile dementia with spastic paralysis (including the pathological examination of a case). *Brain* 63:237-254.

135. Worster-Drought C, Hill TR, McMenemey WH (1933) Familial presenile dementia with spastic paralysis. *J Neurol Psychopathol* 14:27-34.

136. Yamaguchi H, Ishiguro K, Sugihara S, Nakazato Y, Kawarabayashi T, Sun X, Hirai S (1994) Presence of apolipoprotein E on extracellular neurofibrillary tangles and on meningeal blood vessels precedes the Alzheimer beta-amyloid deposition. *Acta Neuropathol (Berl)* 88:413-419.

137. Yamaguchi H, Yamazaki T, Lemere CA, Frosch MP, Selkoe DJ (1992) Beta amyloid is focally deposited within the outer basement membrane in the amyloid angiopathy of Alzheimer's disease. An immunoelectron microscopic study. *Am J Pathol* 141:249-259.

138. Yasuda M, Maeda K, Ikejiri Y, Kawamata T, Kuroda S, Tanaka C (1997) A novel missense mutation in the presenilin-1 gene in a familial Alzheimer's disease pedigree with abundant amyloid angiopathy. *Neurosci Lett* 232:29-32.

139. Zlokovic BV, Ghiso J, Frangione B (2000) Vascular transport of Alzheimer's amyloid b peptides and apolipoproteins. In: *Cerebral Amyloid Angiopathy in Alzheimer's Disease and Related Disorders*, Verbeek MM, de Waal RMW, Vinters HV (eds.), Chapter 20, pp. 325-346, Kluwer Academic Publishers, Dordrecht.

140. Zlokovic BV, Ghiso J, Mackic JB, McComb JG, Weiss MH, Frangione B (1993) Blood-brain barrier transport of circulating Alzheimer's amyloid beta. *Biochem Biophys Res Commun* 197:1034-1040.

141. Zlokovic BV, Martel CL, Matsubara E, McComb JG, Zheng G, McCluskey RT, Frangione B, Ghiso J (1996) Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers. *Proc Natl Acad Sci U S A* 93:4229-4234.